A STUDY OF SERUM COPEPTIN LEVEL IN ACUTE MYOCARDIAL INFARCTION

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This is to certify that dissertation titled **"A STUDY OF SERUM COPEPTIN LEVEL IN ACUTE MYOCARDIAL INFARCTION"** is a bonafide work done by **Dr.S.SASIKALA** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2014 to 2017.

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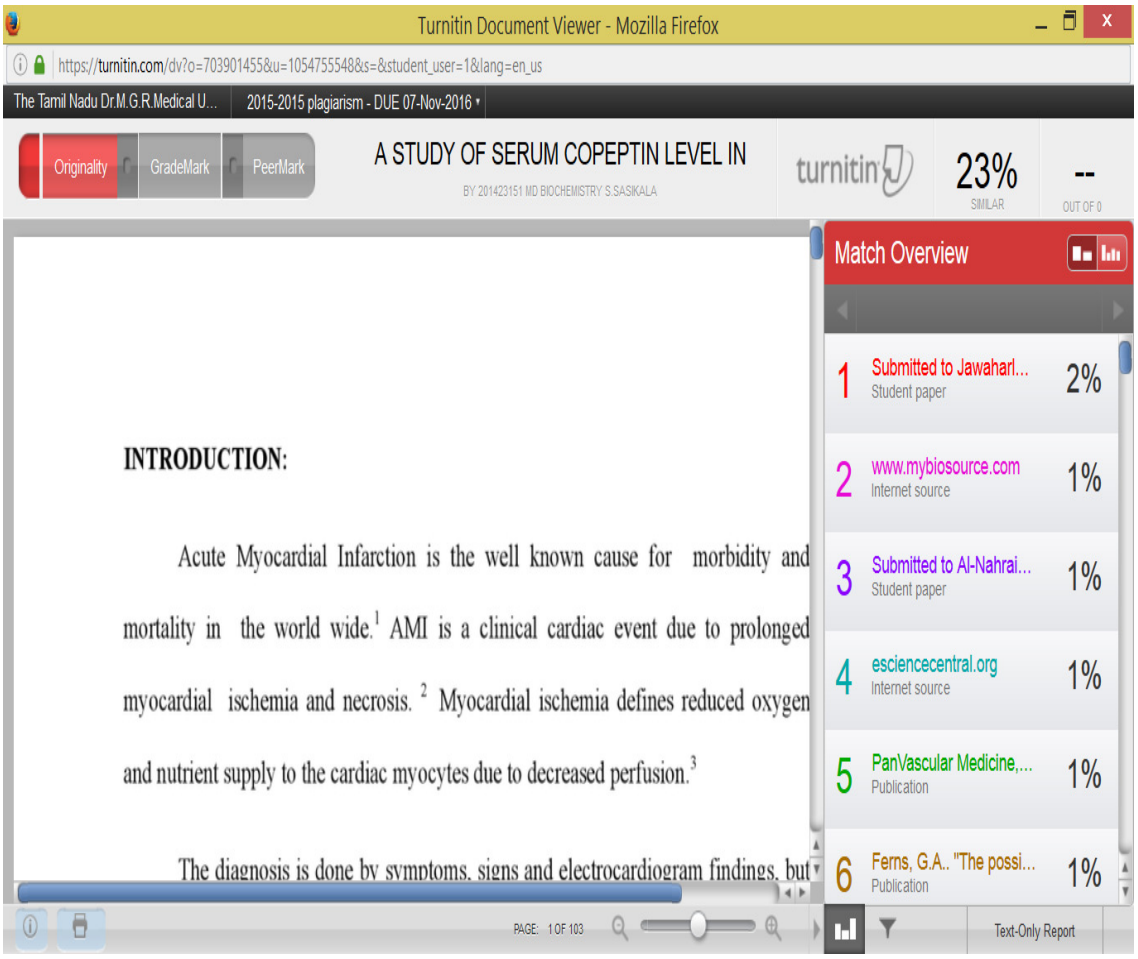

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CONTENTS

ABBREVIATIONS

AMI-Acute Myocardial Infarction

ACS-Acute Coronary Syndrome

RBS –Random Blood Sugar

TC – Total Cholesterol

TGL – Triglycerides

HDL-C - High density lipoprotein Cholesterol

LDL-C - Low density lipoprotein Cholesterol

VLDL - Very low density lipoprotein

CK-MB-Creatine Kinase-MB isoform

IHD – Ischemic Heart Disease.

CHD – Coronary Heart Disease

CVD – Cardio Vascular Disease

CAD-Coronary Artery Disease

MI-Myocardial Infarction

IMA – Ischemia Modified Albumin

ECG – Electro Cardiogram

WHO – World Health Organization

UA-Unstable Angina

STEMI-ST Elevation Myocardial Infarction

NSTEMI-Non ST Elevation MI

PCI-Percutaneous Coronary Intervention

CABG-Coronary Artery Bypass Grafting

CKD-Chronic Kidney Disease

GFR-Glomerular Filtration Rate

BMI-Body Mass Index

CRP-C-Reactive protein

t-PA-Tissue Plasminogen Activator

ESR-Erythrocyte Sedimentation Rate

NO-Nitric Oxide

MPO-Myeloperoxidase

MMP-Matrix Metallo Proteinase

PAPP-A-Pregnancy Associated Plasma Protein-A

H-FABP-Heart –Fatty Acid Binding Protein

BNP-Brain Natriuretic Peptide

FFA-Free Fatty Acid

VCAM-1-Vascular Cell-Adhesion Molecule-1

ICAM-1-Intra Cellular Adhesion Molecule-1

HSP-60-Heat Shock Protein-60

PON-I-Paraxonase-I

cTN I-Cardiac Troponin I

cTN T-Cardiac Troponin T

CT-Pro-AVP-C-Terminal part of Pro-Arginine Vasopresssin

AVP-Arginine Vaso Pressin

ACTH-AdrenoCorticoTropic Hormone

CDC-Centre for disease Control and Prevention

AHA-American Heart Association

ATP-Adenosine Tri Phosphate

ADP-Adenosine Di phosphate

HPA-Hypothalamic –Pituitary-Adrenal axis

ELISA-Enzyme Linked Immuno Sorbent Assay

INTRODUCTION

Acute Myocardial Infarction is the well known cause for morbidity and mortality in the world wide.[1] AMI is a clinical cardiac event due to prolonged myocardial ischemia and necrosis.[2] Myocardial ischemia defines reduced oxygen and nutrient supply to the cardiac myocytes due to decreased perfusion.[3]

The diagnosis is done by symptoms, signs and electrocardiogram findings, but in some patients, it is not possible.[2] Rapid diagnosis of these patients helps to direct further management.[4] Early diagnosis leads to proper treatment in time and also it prevents complications, thereby improving patients outcome.[5]

The most common cause of myocardial infarction is atherosclerosis.The main factors of atherosclerosis are hypertension, diabetes mellitus, hyperlipidemia, smoking, age and gender. The major factors in the initiation of atherosclerotic plaque formation are endothelial dysfunction and inflammation.[6]

The pathogenesis of Atherosclerosis starts with lipid accumulation in the vessel wall and it leads to formation of atherosclerotic plaque which consists of central lipid core surrounded by lipid laden macrophages and smooth muscle cells which is covered by the fibrous cap. The rupture of this cap leads to formation of canal between atherosclerotic plaque and the blood flowing through an arterial lumen.The tissue factor of macrophages activates the thrombocytes, leading to formation of a thrombus.

Finally, the coronary artery is occluded by thrombus, which reduces blood supply to the myocardium leading to ischemia and necrosis and so myocardial infarction.[6] The characteristics of an ideal marker of cardiac necrosis are cardiac specificity, early and stable release after necrosis, predictable clearance that should be easily measured by affordable cost.[1]

Cardiac biomarkers namely, Cardiac Troponin and CK-MB are available for detection of myocardial necrosis. These markers are released only after myocardial necrosis occurs. Delay in release of these markers affects the management. Therefore, cardiac markers with pathophysiology independent of myocardial necrosis might improve rapid diagnosis of AMI.[7,8]

COPEPTIN (CT-pro-vasopressin) is a new recently tested bio marker of acute endogenous (haemodynamic) stress.[9] COPEPTIN is 39 amino acids glycopeptide and secreted with arginine vasopressin (AVP) and it is released from the hypothalamus together in stoichiometric pattern from stimulation of AVP release.[10]

The present study aims to evaluate the serum levels of COPEPTIN as an early marker in AMI,since AMI is an acute stress condition.

REVIEW OF LITERATURE

Ischemic heart disease (IHD) is defined as a condition with inadequate blood flow and oxygen supply to the myocardium.It was mainly due to imbalance between oxygen supply and its demand of myocardium.[3]

Recent definition of Acute MI is existence of myocardial necrosis with clinical symptoms of chest pain and typical ECG changes, such as changes in ST-T wave,new formation of left bundle branch block,and pathological Q waves.[11]

EPIDEMIOLOGY:

More deaths and disability occurs due to IHD with greater loss of economic cost in the developed world.[3] According to World Health Organization , global report in 2011, 7.2 million deaths were caused by AMI or other ischemic disease of the heart.[11]

Risk factors for IHD increases rapidly in the developing world and associated risk factors are smoking, genetic factors, sedentary lifestyle, high-fat and energy-rich diet. IHD will be the most common cause of death in worldwide by 2020.[3]

ANATOMY OF THE HEART:

The heart is a hollow muscular organ,resembling a blunt cone and size of the heart is one's own fist. It is located in the middle mediastinum,between the lungs.

It is covered by a layer called pericardium.The cardiac wall consists of three layers namely, Endocardium,Mesocardium and Pericardium. The heart has 4 chambers namely,two atria and two ventricles.

BLOOD SUPPLY OF THE HEART:

The human heart is supplied by two arteries namely- right and left coronary arteries which is originating from the aorta.They provide nutrients to the heart. These arteries gives rise to many branches from epicardium to endocardium. Among these, endocardium is more susceptible to ischemia because its perfusion depends mainly on smallest branches of the coronary arteries.

The myocardium consists of bundles of striated muscle fibres.It mainly works by alternate contraction and relaxation of muscle fibres. These muscle fibres have specific contractile proteins such as actin and myosin, and regulatory proteins called troponins. Also it has muscle enzymes such as creatine kinase, lactate dehydrogenase, myoglobin and these can be used for detecting cardiac injury.[12]

CARDIAC METABOLISM:

The heart needs a continuous supply of ATP to perform its normal pumping actions, regulating intracellular and trans sarcolemmal ionic movements and also for maintaining concentration gradient. Among its pumping functions, frequency of contraction, development of tension and myocardial contractility are the main determinants of the heart's substantial energy needs. This energy (ATP) production depends mainly on glucose and oxidation of free fatty acids.[13]

MYOCARDIAL OXYGEN SUPPLY:

The supply of oxygen to the myocardium was influenced by coronary blood flow and its oxygen carrying capacity.The coronary blood flow regulation is a complex process and it depends upon several factors.[14]

The factors are

1.Autoregulation

2.Metabolic control

3.Extra vascular compressive forces

4.Diastolic phase in cardiac cycle

5.Neural control

6.Humoral factors

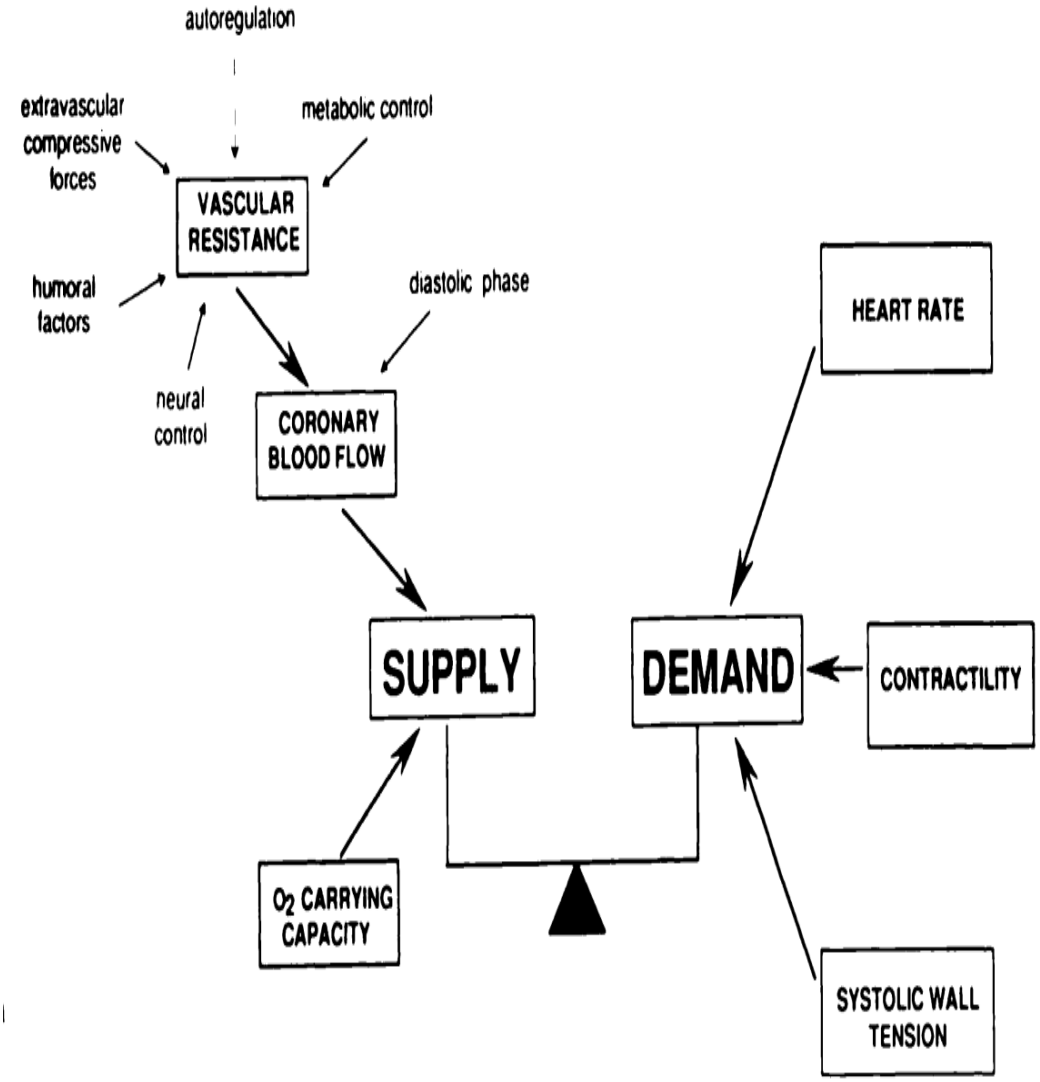
MYOCARDIAL OXYGEN DEMAND:

The heart mainly depends on aerobic metabolism for energy production .The following three important factors influences myocardial oxygen demand

1.Systolic wall tension.

2.Heart rate.

3.Velocity of contraction.[14]



CLASSIFICATION OF IHD:

IHD can be classified into [3,15]

1.Acute Coronary Syndrome-Classified into Acute Myocardial infarction(AMI) and Unstable Angina(UA)

2. Angina pectoris or Chronic stable angina

3.Silent myocardial ischaemia or Chronic ischaemic heart disease

4.Sudden cardiac death.

ACUTE CORONARYSYNDROME:

Acute coronary syndrome is a unifying term denoting a common end result,acute myocardial ischemia.The causes are rupture,erosion or fissuring of an atherosclerotic plaque which can occur alone or in addition with intracoronary thrombosis.Thereby it leads to increased risk of myocardial necrosis.[16]

Acute Coronary Syndrome (ACS) encompasses a broad range from the patients with atypical chest discomfort, nonspecific ECG findings, and normal cardiac biomarkers to the patients with AMI [ST-segment Elevation Myocardial Infarction (STEMI), Non-ST-elevation Myocardial Infarction (NSTEMI) and Unstable Angina[UA].[17]

ACUTE MYOCARDIAL INFARCTION:

Acute Myocardial Infarction is assessed by clinical features, ECG findings, biomarkers and also by cardiac imaging.[17]

CLASSIFICATION OF AMI:

Acute Myocardial Infarction is classified into many types, based on clinical, pathological and prognostic differences,along with its various treatment strategies.

On the basis of ST segment elevation,AMI is classified into

1.STEMI-ST-segment Elevation MI or Transmural MI

2.NSTEMI-Non-ST-segment Elevation MI or Sub-endocardial MI

ST-SEGMENT ELEVATION MI / STEMI OR TRANSMURAL MI:

In STEMI ,myocardial necrosis is assessed by either ECG changes with ST-segment elevation usually not rapidly reversed by nitroglycerin or evidenced by the presence of new left bundle branch block and Q waves may possibly be present. Specific Cardiac markers such as CK-MB and troponin T are found to be increased.

NON ST-SEGMENT ELEVATION MI / NSTEMI OR

SUB-ENDOCARDIAL MI:

In this condition there is elevated levels of cardiac biomarkers such as troponin T and CK-MB but without the formation of Q waves or acute ST-segment elevation. But ECG changes like T-wave inversion, ST-segment depression,or both may be present.

Based on etiology and circumstances, MI can be classified into five types:

Type 1: After rupture of an atherosclerotic plaque or erosion or fissuring or coronary dissection which results in spontaneous Myocardial Infarction.

Type 2: Myocardial ischemia is caused by increased O_2 demand (e.g hypertension) or reduced oxygen supply due to embolism, coronary artery spasm or arrhythmia or hypotension

Type 3: Due to unexpected sudden cardiac death

Type 4a: Myocardial Infarction during Percutaneous Coronary Intervention(PCI)

Type 4b:Myocardial Infarction due to Thrombosis of documented stent .

Type 5: Myocardial Infarction after the procedure of Coronary Artery Bypass Grafting (CABG).[18,19]

ANGINA PECTORIS:

Angina pectoris is one of the important presentation of Coronary Heart Disease.The symptoms are feel of compression in the precordial region and discomfort in the chest which occurs mainly due to temporary disruption of blood flow to the cardiac musculature not progressing to overt infarction. Symptoms are aggravated during exercise and mental stress and it is relieved by taking rest and sublingual nitroglycerin.[20,21]

UNSTABLE ANGINA:

It is also known as preinfarction angina or acute coronary insufficiency or intermediate coronary syndrome. Chest discomfort is similar to that of classic angina,but its intensity is more. Reduction in physical activity threshold, is a warning sign for the onset of angina. In addition to this, nocturnal angina or rest angina will occur. Transient ECG changes like depression or elevation of ST segment, or depression of T wave can occur in unstable angina. ECG monitoring should be done to find arrhythmias with sudden onset & to detect the abnormalities of ST segment for diagnosing recurrent ischemia.[21]

PRINZMETAL ANGINA:

Prinzmetal angina (variant angina, angina inversa, or coronary vessel spasm) is another presentation of myocardial ischemia ,which is characterised by chest pain during rest hours (angina) and it usually occurs in cycles. In this type,the cause of chest pain is due to vasospasm, a narrowing of the coronary arteries mainly due to contraction of smooth muscles in the blood vessels. [22]

The following are the risk factors for cardiovascular disease .[23,24]

MODIFIABLE RISK FACTORS :

Hyperinsulinemia and insulin resistance.

Hypertension

Obesity

Hyperlipidemia

Physical inactivity

Tobacco use

PARTIALLY MODIFIABLE RISK FACTORS:

Chronic Renal Disease

Post menopausal oestrogen deficiency

Diabetes

Stress

Infection

Socioeconomic status

Personality

Microalbuminuria

NON-MODIFIABLE RISK FACTORS:

Race

Age(female ≥55 years,male≥ 45 years)

Gender

Genetic factors

Low birth weight.

Family history.

Previous history of coronary artery disease

SELECTED EMERGING RISK FACTORS

C-reactive protein

Small LDL particles

Lipoprotein (a)

Homocysteine

Lipoprotein –associated phospholipase A2

Apolipoproteins A and B

HYPERTENSION:

There is risk of Cardiovascular disease with rising systolic and diastolic blood pressure. Serum cholesterol is one of the continuous variables and there is no comprehensible cut-off value, but hypertension increases the risk of CHD at any given value of cholesterol. Because intra subject blood pressure measurements can

vary over time , the diagnosis of hypertension relies on the measurement of blood pressure on several occasions. Also,hypertension is associated with obesity and dyslipidemia, leading to increased risk of CHD.[25]

INSULIN RESISTANCE AND HYPERINSULINEMIA:

The term Insulin resistance defines decreased rate of glucose uptake which is mediated by insulin. In these conditions,there will be increased levels of insulin in the body and undesirable cardiovascular risk factors such as increased triglyceride level and decreased HDL cholesterol. Cross-sectional studies have proven that insulin resistance is linked with atherosclerosis which has ultrasonographically proved in the absence of hyperlipidemia and hypertension.[26]

INSULIN RESISTANCE AND HYPERTENSION:

The concurrence of Hypertension and Insulin resistance doubles the risk of Cardiovascular complications. In normal individuals, insulin induces the endothelial cells to increase the production of Nitric Oxide, which is a potent vasodilator.[27] Thus, Insulin resistance causes defect in vasodilatation leading to hypertension.[28]

DIABETES MELLITUS :

Many patients with Acute Coronary Syndromes (ACS) coming to hospital are associated with hyperglycemia. Hyperglycemia is very important to predict the survival and risk of complications in both diabetics and non-diabetics. When compared to patients admitted with normal blood glucose concentration, there is 70% rise in relative risk in patients with blood sugar level of 180 mg/dl.Impaired

glucose tolerance and diabetes are most important risk factors for CVD and epidemiological data have led to the notion that diabetes confers a similar risk of a cardiovascular event to a prior Myocardial Infarction. Diabetic patients with increased haemoglobin A1c (HbA1c) levels is possibly related to an increased CVD risk.[29]

METABOLIC SYNDROME AND CVD:

Individuals with metabolic syndrome are at increased risk of CVD and total mortality, especially in males aged 45 years and females aged 55 years.[30,31] And those with frank diabetes or prior CVD are at a even highest risk[30]. Concurrent occurrence of hypertension and impaired glucose metabolism has the highest risk.[32] Individuals with atherosclerosis and metabolic syndrome without diabetes are at increased risk of CVD and total mortality.[33]

In the patients with IHD, Metabolic syndrome prevails in almost 50%. Life style changes and the cardiac rehabilitation can lower the prevalence of this syndrome.[34] Both obese and non-obese individuals with metabolic risk factors should be targetted to bring down the burden due to CVD in normal population.[35]

Population dependent preventive steps must be undertaken by increasing the awareness of the association of risk factors, in particularly for South Asians.[36] Epidemiologists from India and international agencies like WHO (World Health Organization) proposed the rapidly rising impact of CVD as a potential danger for the past 15 years. The current estimate is about 2.6 million among Indians, by 2020,CVD being the commonest cause of morbidity and mortality.[37]

DYSLIPIDEMIA AND CORONARY ARTERY DISEASE:

The risk factor for Coronary artery disease in the metabolic syndrome is the existing Dyslipidemia. The main cause for Dyslipidemia is excessive synthesis of apolipoprotein B containing VLDL (very low density lipoprotein) particles.[27] The physiological function of Insulin is to suppress VLDL production, in particular Apolipoprotein B particles, in the liver, by the following mechanisms:

1. Decreases FFA availability by inhibiting lipolysis in adipose tissue.[38]
2. Direct hepatic effect - by affecting the assembly and hence the synthesis of VLDL.[39, 40]

In skeletal muscle, Insulin resistance is directly related to the intra-myocellular level of Triglycerides.[41] So, the patients with metabolic syndrome are subjected to aggressive treatment with lipid lowering agents to prevent cardiovascular complications.[27]

LIPIDS AND LIPOPROTEINS:

The association between plasma lipids and coronary heart disease is positively correlated in various studies and it was continuous, exponential and showed no threshold ,even at lesser values.The total cholesterol concentrations among patients with and without CHD overlap to a considerable amount. Plasma apolipoprotein B (apo B) concentrations are more discriminating and a few clinical studies have shown that apo B concentrations is the basis for therapeutic management.

An inverse relationship is seen between plama HDL concentrations and CHD risk has also been seen in various studies. Clinical studies on protective effects of HDL with animal models, in which expression of human apo-AI has been studied to inhibit the development of atherosclerotic lesions. The beneficial effect of HDL is due to its antioxidant properties associated with an intrinsic paraxonase I (PON I) activity.[42]

Low PON I activity is an self-sufficient predictor of new coronary events and its polymorphism is reported to be associated with increased lipid peroxide hydrolysis. Recent data do support a role for triglycerides as an independent risk factor. Plasma triglycerides concentration higher than 1.7mmol/L leads to development of more atherogenic,small,dense LDL and also it has proatherogenic effects by promoting a procoagulant state, being correlated with enhanced factor VII activity.[43]

HYPER TRIGLYCERIDEMIA:

Following the hydrolysis of exogenously derived chylomicrons or endogenously secreted VLDL, cholesterol enriched remnant by- products enter the sub-endothelial space. In this condition,there is an oxidative and proinflammatory milieu enhancing expression of adhesion molecule, formation of foam cell and intoxication of smooth muscle cell. Hypertriglyceridemia reduces reverse cholesterol transport. It has been shown that 10% lowering of TGL concentration decreases the risk of CHD by 23% .[44]

CHRONIC KIDNEY DISEASE(CKD):

Patients with CKD are easily prone to develop arteriosclerosis and large arteries remodelling due to vascular calcification. Decreased Glomerular Filtration Rate (GFR) have relationship with a CVD risk factors, higher CVD surrogates and clinical patients. Recently, the extent of demonstrable angiographic coronary disease reflects the stage of kidney function in patients with CKD. For example,female with chest pain undergoing angiography with increased creatinine value of 1.2 to 1.9 mg/dL is an self-determining predictor of significant angiographically proved coronary disease, with luminal narrowing of 50%.[45]

OBESITY:

Obesity is an important risk factor for CHD and also growing in prevalence throughout world wide. Body mass index is positively related to fasting triglycerides levels, plasma cholesterol and blood pressure, and inversely related to HDL- cholesterol level. Therefore, it is the central or visceral obesity , measured as waist circumference,that is associated to insulin resistance and CHD risk. Waist circumference is a significantly better index of insulin resistance than either waist/hip ratio or BMI. A cut off value for waist circumference of <100 cm rules out insulin resistance in both male and female with optimal sensitivity and specificity. If the weight gain is more, there is high risk of insulin resistance,diabetes, hypertension and ischemic heart disease . Body mass index of> 30 Kg/m^2 is defined as “obesity” and it plays most important role in the progression of an atherosclerosis.[46,47]

LOW PHYSICAL ACTIVITY :

Low physical activity and sedentary life style leads to development of CHD. Routine physical exercise increases the level of S.HDL and decreases both body weight and blood pressure which is most beneficial to cardiovascular health. About 30min/day of moderate exercise is enough to have a significant impact on coronary risk. For patients with CHD,exercise based rehabilitation is helpful in decreasing total mortality and also lipid levels.[48]

SMOKING

Cigarette smoking is one of the well known independent risk factor of fresh coronary lesion. A change in NO biosynthesis results in both primary and secondary effects on the initiation and progression of atherosclerosis. The important risk factors are smoking, malnutrition and lack of exercise which contributes to occurrence of coronary heart disease.[49,50]

The increased susceptibility for the smoking associated atherosclerosis and multi vessel CAD was due to CYP1A1 MSP polymorphism and certain endothelial NO synthase intron 4 polymorphisms. Cessation of smoking is effectual in secondary prevention of the coronary heart disease. A recent study has shown that continuous 3 to 7 years after cessation of smoking in patients with known coronary heart disease led to 30% decrease in crude risk rate of death and Acute Myocardial Infarction (AMI). [25]

ALCOHOL INTAKE:

Recent studies have shown that consumption of alcohol more than 89 g/day results in high prevalence of CVD.[51] Alcohol consumption rises the level of plasma lipid parameters like triglycerides, VLDL and the large, buoyant LDL subfraction , rather than small, dense LDL which contribute to a proatherogenic state.

AGE:

Increasing prevalence of atherosclerosis among young individuals may be due to rise in cholesterol level and other coronary risk factors such as age . The relative risk is steeper with increasing levels of cholesterol in young individuals. The prevalence of aortic and coronary atherosclerosis increases with age.[52]

GENDER:

Women with Acute Myocardial Infarction are mostly older than men and also with a history of diseases namely diabetes mellitus, hypertension, angina & congestive heart failure. Women has a higher percentage of S.HDL cholesterol than men and it is due to the action of oestrogen and also it is vascular protective. In post menopausal period,there is rise in plasma LDL cholesterol,fall in S.HDL cholesterol and increasing visceral adiposity which leads to increase in risk for CHD. The relative protection in female gender becomes low,when they have diabetes mellitus.[52,53,54,55]

FAMILY HISTORY:

A family history of premature CVD is one of the important risk factors. Familial tendency to atherosclerotic lesions and ACS are always certainly polygenic. In females, age-adjusted risk is increased almost three fold if either parent has CHD before 60 years of age. Moreover risk factors like Diabetes mellitus (DM) and Hypertension are more inclined to genetic susceptibility. [55]

STRESS:

Mental stress is one of the important precipitating factors for angina and myocardial ischemia in patients with coronary artery disease. Myocardial oxygen demand is enhanced by sympathetic activation and it is characterised by high blood pressure,tachycardia and increase in myocardial contractility which further enhances the myocardial oxygen demand. Although stress induces increased blood flow, there will not be adequate increase in coronary blood flow in the presence of atherosclerosis inspite of increase in the product of blood pressure & heart rate and increase of plasma norepinephrine levels.[56]

THOMBOGENESIS AND CLOTTING FACTORS:

Thrombin uses fibrinogen as the substrate for the final stage in cascade of coagulation. It is necessary for platelet aggregation,modulation of endothelial function to promote smooth muscle proliferation. Plasma fibrinogen concentration is strongly associated with CHD. The relationship between plasma fibrinogen and coronary risk may underlie the positive association between plasma viscosity and CHD.[57]

The tissue factor is released due to injury to endothelium which in turn, activates the intrinsic clotting cascade. Platelet activation and aggregation are important crucial processes in athero-thrombogenesis, and platelet reactivity has been reported to be increased in patients with diabetes and unstable angina. There is a balance between the formation of clot and its inhibition and dissolution by factors such as proteins C and S and plasmin. The fibrinolytic system based on the balance between tissue plasminogen activator (tPA) and inhibitors of plasminogen activator,which converts plasminogen to plasmin, which mainly acts on fibrin causing clot dissolution. This process is inhibited by PAI-I , high concentrations of which are associated with increased risk for reinfarction.

Apolipoprotein(a) is a glycoprotein that has structural homologies with plasminogen. It is attached to apo B by a disulphide bond and in some individuals, comprises the major cholesterol rich lipoprotein. The structural similarities between apo(a) and plasminogen level have led to the proposition that Lp(a) inhibits plasmin activity, leading to a prothombotic state. Plasma concentration of Lp(a) are largely genetically determined, but can be modified to a limited degree, by dietary fatty acids, oestrogen and lipid lowering agent like nicotinic acid.

HYPERHOMOCYSTEINEMIA:

Homocysteine is one of the sulphur containing aminoacid, derived mainly from methionine, the concentration of which is changed by dietary vitamin B6, B12 and folate. The normal plasma homocysteine level is 5-15μmol/L. Very high concentration of homocysteine (> 100 μmol/L) is injurious to endothelial cells and contributes to premature vascular disease due to free radical mediated mechanism. High concentration of homocysteine inhibits Nitric Oxide (NO) indirectly by stimulating superoxide anion production from endothelial cells. Also, it elevates the atherogenecity of Lp(a) by liberating free apo(a) impeding fibrinolysis. There is a dose dependent increase in cardiovascular risk with increasing plasma homocysteine concentration, synergistic with other coronary risk factors and a number of established coronary risk factors are associated with high levels of homocysteine, including smoking and renal impairment.[26]

INFLAMMATION AND INFECTION:

Atherosclerosis involves mechanisms similar to a chronic inflammatory disease, the course of its evolution being characterised by T cells and macrophage permeation. The stimuli to inflammatory process include homocysteine, oxidized LDL , infectious micro-organisms and the free radicals which is generated from cigarette smoking.In the absence of neutralisation of insults, persisting inflammation results, which may cause local and systemic release of cytokines and growth factors. This is followed by thickening of the intimal layer due to

stimulation of extracellular matrix elaboration and smooth muscle cell migration and proliferation. Thus,activated leucocytes release IL- 1β and IL-6 which leads to increase in hepatic CRP synthesis.

The role of infectious micro organisms in development of atherosclerosis is debatable. Over the past years, micro organisms such as Helicobacter pylori and Chlamydophila pneumoniae were known to play important role in the pathogenesis of atherosclerosis. Treatment of patients with antibiotics might decrease the risk of atherosclerosis. These micro-organisms also found in the plaques in human beings and those with Acute Coronary Syndrome has higher titers of antibodies to these organisms .

Autoimmune mechanism has also been involved in the pathophysiology of atherosclerosis. Some micro-organisms elaborate proteins called chaperones or heat shock proteins-60(Hsp-60).These are homologous to a protein ,which is synthesised in the vascular endothelium. It is postulated that an immune responses mounted against this protein may react with endothelial heat shock proteins resulting in injury to the endothelium. Autoimmunity has been postulated as the link between atherosclerosis, endothelial dysfunction and infection. Herpes,Chlamydia and Cytomegalovirus are some of the infectious organisms suspected to contribute to atherosclerosis.[58]

PATHOPHYSIOLOGY OF ACUTE CORONARY SYNDROME:

In ACS,atherosclerotic plaques and non-stenotic lesions are more commonly implicated rather than the coronary artery stenosis. Non occlusive plaques become complicated by thrombus and progresses rapidly to complete occlusion.

ACS is due to five main causes:

1.Plaque rupture with acute thrombosis by formation of blood clot that gradually occludes the lumen ,which is the important event in atherothrombotic process.

2.Progressive increase of plaque volume leads to mechanical obstruction.

3.Inflammation by growth factors, enzymes &local and systemic production of cytokines activate the plaque and disrupt the fibrous cap.

4.Coronary vasoconstriction occurs due to dynamic obstruction influenced by endothelial dysfunction.

5.Plaque embolization occurs at distant level from atherothrombotic coronary occlusion.[59]

In most cases of ACS,sudden luminal thrombosis is a consequence of either plaque rupture ,plaque erosion, or a calcified nodule.Prolonged chest pain at rest often results from fissuring or rupturing of the atherosclerotic plaque leading to thrombus formation.Plaques are prone to rupture and contain large amounts of extra cellular cholesterol covered by a thin cap of fibrotic tissue.

The immediate site of plaque rupture is marked by an inflammatory process (macrophage rich area).The inflammation plays a role in destabilizing the fibrous cap tissue. Factors predisposing to plaque rupture are circulating immune complexes,nicotine,hyperlipidemia, high angiotensin level, elevated acetoacetic acid level in diabetes.The junction between the normal and atherosclerotic segments of an eccentric stenosis is the site for rupture of the fibrous cap. [60,61]

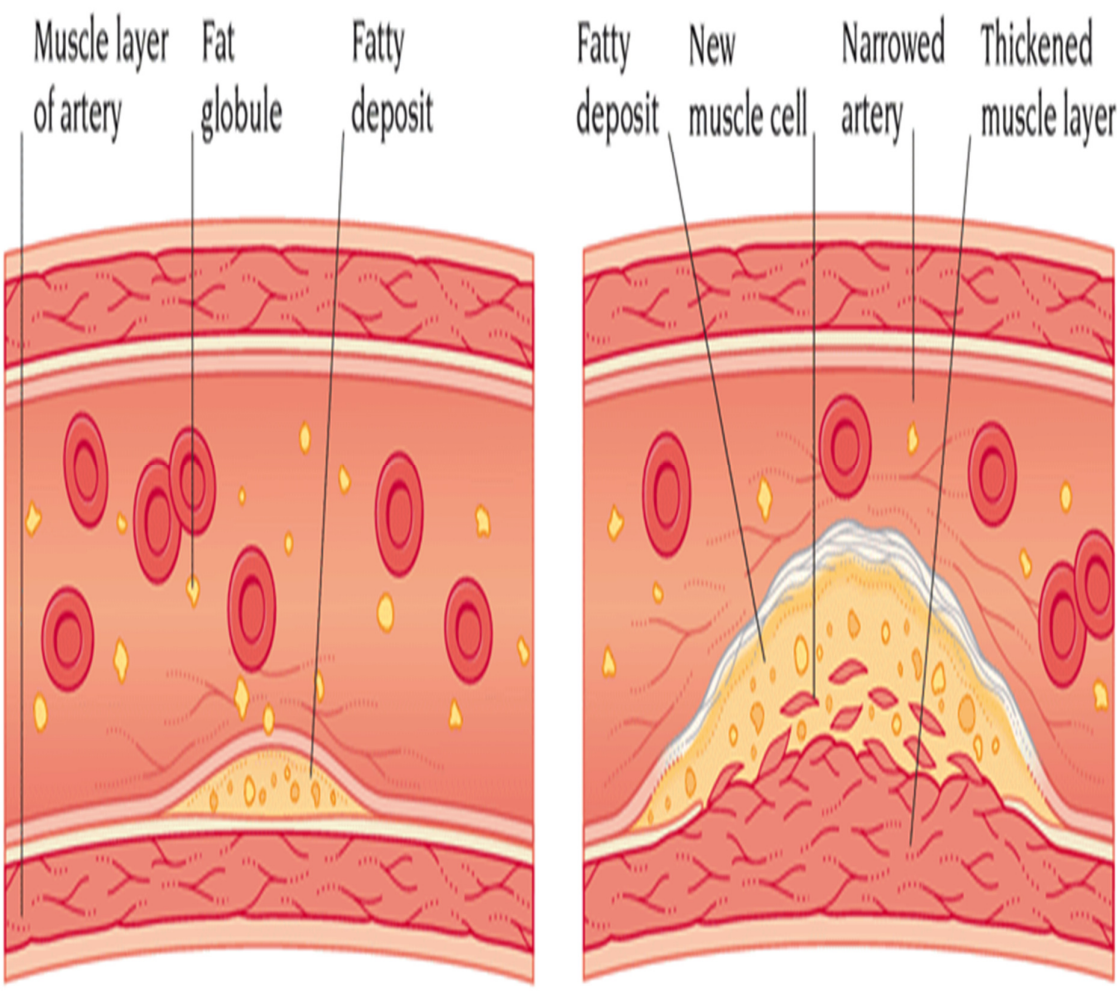
ATHEROSCLEROSIS:

Atherosclerosis is a chronic disease of large and medium sized arteries, which results in progressive accumulation of inflammatory cells, smooth muscle cells, lipid and connective tissue within the tunica intima.[62]

LESION INITIATION, PROGRESSION AND COMPLICATIONS OF ATHEROSCLEROTIC PLAQUE:-

Fatty streak is the earliest lesion of atherosclerosis, made up of lipidladen foam cells, derived mainly from circulating monocytes and few smoothmuscle cells. Foam cells represent circulating monocytes, that penetrate between the endothelial cells, enter the intima and are taken up lipoproteins and consequently stored as cholesterol esters in multiple droplets.[63]

ATHEROSCLEROSIS



EARLY ATHEROSCLEROSIS

ADVANCED ATHEROSCLEROSIS

Atherosclerotic lesions were graded according to the types of lesion by the American College Of Cardiology/American Heart Association Classification.[64]

Type A lesions are defined as discrete lesions(<10mm in length).

Type B lesions are tubular lesions.(10-20 mm in length)

Type C lesions are diffuse lesions (>2 cm in length)

Atherosclerosis represents a chronic inflammatory response to vascular injury and activate endothelium and promote lipoprotein infiltration ,modification and retention combined with inflammatory cell entry,retention and activation.[65] Atherosclerosis can affect any artery .In the heart,due to atherosclerosis it can cause angina,MI and sudden death.In the brain,it may cause stroke and transient ischemic attacks.In upper and lower limbs,it can cause claudication and critical limb ischemia.[66]

PATHOPHYSIOLOGY OF ATHEROSCLEROSIS:

Endothelial injury is the initiating event in atherosclosis. Endothelial injury is caused by hypertension, hypercholesterolemia, local hemodynamic abnormalities, cigarette smoking, hyperhomocysteinemia, increased LDL-C,C-reactive protein, increased fibrinogen, insulin resistance, oxidative stress, infections like Herpes virus or Chlamydia pneumoniae,periodontal disease and combination of these factors.[67,68]

Endothelial dysfunction is due to the accumulation of macrophages.These are derived from lipids and circulating monocytes .In the region of vascular injury,oxidation of low density lipoproteins and their ingestion by macrophages produces foam cells.The foam cells aggregate to form the fatty streak,the earliest lesion of atherosclerosis.The atherosclerosis is a complex disease involving increased pro-oxidant stress,inflammatory fibro-proliferative and angiogenic responses combined with smooth muscle cell proliferation which results in plaque formation.[69]

THEORIES OF ATHEROGENESIS:

Atherogenesis is the major cause of cardiovascular disease,due to the formation of atheroma which leads to arterial hardening.Several theories have been put forward.

THE RESPONSE TO INJURY HYPOTHESIS OF ROSS:

According to this theory,platelets as a source of growth factors,interact with injured epithelium of damaged arterial wall leading to atherosclerosis. Endothelial injury is caused by so many risk factors,such as viral infection,smoking, hyperlipidemia,and hypertension. [70]

THE LIPID OXIDATION HYPOTHESIS OF STEINBERG AND COLLEAGUES:

This hypothesis explains mechanism of endothelial injury and formation of macrophage derived foam cells that are characteristic of the early lesions of atherosclerosis.Leucocytes adhesion to endothelium occurs early.There will be subsequent accumulation of T-lymphocytes and monocytes in sub-endothelial space.Within the arterial wall the monocytes were converted to foam cells and form macroscopically evident fatty streak lesion.The T cells was activated,and it is initially involved in a Th1-type response,associated with a release of proinflammatory cytokines.

An adhesion event is mediated by the pairs of molecules,one of each pair being on the leukocytes,the other on the endothelial cell. The endothelial adhesion molecules have been shown to be upregulated early in atherogenesis and may be modulated by immune stimulation and antioxidant treatment. Excessive uptake of cholesterol by smooth muscle cells and macrophages,sufficient for the formation of foam cells that are characteristics of the fatty streak ,cannot occur via the low density lipoprotein (LDL) receptor pathway. This is due to tight regulation of LDL receptor expression by intracellular cholesterol.

Goldstein and Brown proposed the existence of another 'scavenger receptor' pathway,which permits the unregulated uptake of cholesterol as modified LDL.Cells in an arterial wall can cause an oxidation of LDL in the presence of

transition metal ions,resulting in the release of LDL oxidation products such as malondialdehyde,4-hydroxynonenol and lipid peroxides.

These may inturn react with apo B-100 within LDL particles ,causing cross-linking and altered antigen expression,thus facilitating subsequent recognition and uptake by the scavenger receptors.Epitopes of oxidized LDL was present in atherosclerotic lesions and this LDL isolated from lesions has similar characteristics to oxidized LDL,which cross reacts with antisera raised against malonialdehyde and 4-hydroxynonenol modified LDL and it is recognized by the scavenger receptor.

Inhibiting LDL oxidation in vivo by treatment with an antioxidants such as vitamin E inhibits experimentally –induced atherogenesis.LDL modification also appears to lead to the expression of neo-antigens that elicit an autoimmune response and,indeed,autoantibodies to oxidized LDL have been found in human plasma,and within human atherosclerotic lesions.

COURSE OF ATHEROSCLEROSIS:

Accumulation of low density lipoprotein particles (LDL) in the intima is the initial step leading to fatty streaks.Fatty streaks are formed when inflammatory cells,predominantly monocytes bind to endothelial cell receptors and migrate into the intima.They then develop into lipid-laden macrophages or foam cells by taking up oxidized LDL-C that are universally present in atheromatous plaques .[71,72,73]

Extra cellular lipid pools appear in the intimal space during the death of foam cells which releases their contents. Activated macrophages produces growth factors and cytokines. There is migration of smooth muscle cells into the intima of the arterial wall from the tunica media. Macrophages also stimulate smooth muscle cell proliferation. Oxidized LDL can induce production of Vascular Cell Adhesion Molecule-1(VCAM-1) & Intra Cellular Adhesion Molecule-1(ICAM-1) which can lead to increase in size of the plaque.[74]

Cytokines such as Tumour Necrosis Factor-alpha, interleukin, interferon-γ, platelet derived growth factor and matrix metalloproteinases are released by activated macrophages.They cause degradation of collagen cross-struts within the plaque and senescence of the intimal smooth muscle cells overlying the plaque.[75]

This results in thinning of the protective fibrous cap and there by causing the lesion vulnerable to mechanical stress.This in turn causes fissuring, erosion or rupture of the surface of the plaque. When there is any breach in the integrity,the contents of the plaque are exposed into the blood. This triggers platelet aggregation and thrombosis and extends into the arterial lumen. [76,77,78]

ACUTE CHANGES IN PLAQUE FALL INTO THREE GENERAL CATEGORIES:

1.Rupture/fissuring-exposing highly thrombogenic plaque constituents and subsequent thrombosis.such thrombosis can partially or completely occlude the lumen and lead to down stream ischemia.

2.Erosion/ulceration exposing the thrombogenic sub-endothelial basement membrane to blood.

3.Haemorrhage into the atheroma expanding its volume. Rupture of the overlying fibrous cap or the thin walled vessels in the areas of neovascularisation,can cause intra plaque haemorrhage.A contained haematoma may expand the plaque or induce rupture of the plaque.

Rupture of the plaque can discharge atherosclerotic debris into the blood stream, producing micro emboli.[75] When rupture leads to total thrombotic occlusion in the coronary vessels, the event is usually STEMI. When lesser degrees of occlusion occur, a NSTEMI or UA may ensue.

SYMPTOMS OF AMI [79]

1.Very severe chest pain at sternal region,which is radiating to the jaw and shoulders mostly to one or both arms

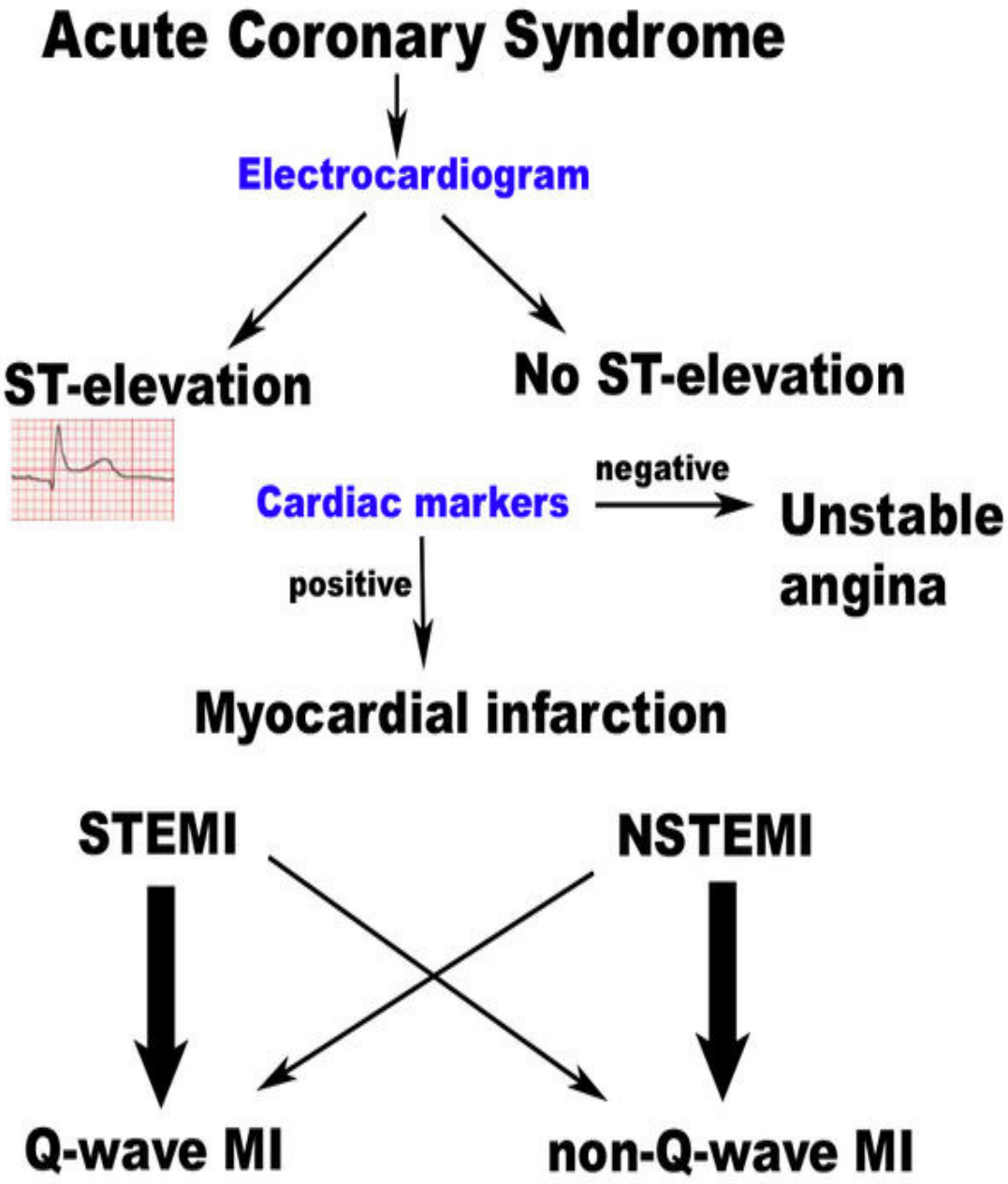
2.Tightness around the chest radiating to the left arm and jaw.

3.Diaphoresis or sweating

4.Vomiting sensation with or without Nausea.

5.Dyspnoea or Shortness of breath.

DIAGNOSIS OF ACS



6.Atypical chest pain

7.Palpitations

8.Anxiety

9.Angor animi (sense of impending doom)

10.Feeling acutely ill.

ECG ABNORMALITIES:

ECG changes occur due to inability of cells affected by ischemia and necrosis to produce normal electrical activity. Various ECG changes like conduction abnormalities, ST- T wave changes and arrhythmias may occur. ST-T abnormalities may range from peaking of T waves in the hyperacute phase, elevation of ST segment, inversion of T wave and ST segment depression.Damage to the specialised conduction tissues like sinus node, AV node and other tissues may result in conduction disturbances. While most of these changes are temporary, some may become permanent.[80]

DIAGNOSIS OF AMI:

1. Criteria of World Health Organization for an Acute Myocardial Infarction (1986): Presence of atleast two of the following

a) Complaints suggestive of coronary ischemia for a prolonged period. (>30 minutes) b) Evolutionary changes on sequential ECG indicative of myocardial infarction. c) An increase or decrease in serum cardiac markers steady with myocardial ischemia or myocardial necrosis.

2. Criteria for the Definition of Acute Myocardial Infarction (European Society of Cardiology/American College of Cardiology (2000 updated in 2007 (Global task force))

Detection of raise and / or decrease in the level of cardiac biomarkers (preferably troponin) above 99th percentile of the upper reference limit along with the findings of myocardial ischemia with atleast one of the following below:

i. Symptoms suggestive of ischemia

ii. ECG changes of fresh ischemia (new ST-T changes or new Left Bundle Branch Block (LBBB)

iii. Evidence of pathological Q waves on ECG

iv. Imaging based evidence of recent loss of viable myocardium or latest regional wall motion abnormality

3. Diagnosis of Established Myocardial Infarction: Anyone of the following criteria satisfies the diagnosis of established MI

a. Appearance of new pathologic Q waves on consecutive ECGs. The patient may or may not recollect the symptoms. Normalised biochemical markers of myocardial necrosis depending on the length of time that has passed.

b. Pathologic findings of a healed or healing MI.

Apart from clinical and ECG findings ,cardiac biomarkers have become indispensable in the diagnosis of AMI.

CARDIAC BIOMARKERS:

Cardiac biomarkers are protein substances released into the bloodstream from the damaged myocardium due to blockage of coronary artery and estimation of these enzymes have a general idea of amount of muscle damage . Many new biomarkers are available and it has more sensitivity and specificity in the diagnosis of acute coronary syndromes.[81]

An increasing number of novel biomarkers have been identified to predict the outcome following AMI. This may facilitate for giving treatment appropriate therapy to high-risk patients.[82] So,cardiac biomarkers plays an important role in the management of CAD disease and acts as cornerstone in diagnosis and prognosis of CAD.[83]

CLASSIFICATION OF BIOMARKERS IN ACUTE CORONARY SYNDROME: [84,85]

ESTABLISHED MARKERS

1. Troponin I
2. Troponin T
3. (BNP)Brain Natriuretic Peptide
4. N - Terminal Pro -BNP
5. C-Reactive protein
6. Cystatin C
7. Heart – Fatty acid Binding Protein.

EMERGING MARKERS:

1. Myeloperoxidase

2. Matrix Metalloproteinase

3. Soluble CD40 ligand

4. Ischemia Modified Albumin

5.Copeptin

6. Pregnancy associated plasma protein-A

CARDIAC TROPONINS :

Cardiac troponins are most specific than other cardiac biomarkers for detecting myocardial injury.Following AMI, cardiac troponin I (cTnI) will increase at the same rate as CK-MB. It peaks at 12-24 hours, and remains elevated for the period of 7-10 days. Cardiac troponin T (cTnT) is seen within 3 to 6 hours following the symptoms of onset. Like cTnI, it remains in the blood for 7-10 days after injury.[86]

CREATINE KINASE – MB:

Creatine Kinase (CK) which is a muscle enzyme exists as three isoforms. CK-MM (skeletal muscle), CK-MB(myocardium) and CK-BB (brain). The pathophysiological basis for increase in CK-MB is that a state of myocardial hypoxia which causes heart to respond to increased workload by increasing its mass and thus leading to elevation of CK-MB.The level of CK-MB will be elevated after 3 hrs of chest pain.[87]

MYOGLOBIN

Myoglobin is a single polypeptide (153 amino acids) heme protein found in the cytosolic fraction of both skeletal and cardiac muscle . It was catabolised by glomerular filtration, proximal renal tubular absorption through the process of endocytosis and proteolysis. Currently,Myoglobin is the earliest biological marker of myocardial necrosis. Its molecular weight is 17.8 kiloDalton and it is low.So,it gives a quicker diffusion in the circulating blood than enzymes such as CK or LDH. Myoglobin is released in the peripheral blood within 3 hrs after chest pain.The peak level is attained within 6-9 hrs of chest pain and it returns to normal level within 18-36 hours.[88]

B- TYPE NATRIURETIC PETIDE (BNP)

It is secreted by ventricular myocardium in responding to the stress of ventricular wall,including volume expansion and pressure overload.Many studies have shown that BNP is one of the prognostic indicator in acute coronary syndrome.[81]

MYELOPEROXIDASE (MPO):

Inflammatory cells like activated neutrophils and monocytes inside the atherosclerotic plaque produces MPO which acts as a mediator enzyme. MPO is known to induce oxidative damage of tissues by generating reactive oxidised intermediates. Low density lipoprotein (LDL) isolated present inside the

atherosclerotic lesions found to have increased levels of such intermediates. MPO expression isincreased at the site of plaque rupture,superficial erosions and in the lipid core in patients followed with sudden death. Whereas MPO expression levels were found low in fatty streaks of less harm.[89]

C-REACTIVE PROTEIN:

CRP is an acute phase reactant produced in response to interleukin (IL-6)in the liver . It is very stable pentameric protein and its concentration in the serum can increase many folds during the acute phase response. In January 2003, CRP was recommended as an inflammatory marker for recognition of cardiovascular risk by both the Centre for disease Control and Prevention (CDC) and also by the American Heart Association (AHA). Identification and stratification of patients used Cardiac CRP (cCRP) assays to assess the future CVD associated events.[90]

SOLUBLE CD40 LIGAND:

sCD40 ligand is the signal protein points to both inflammatory and platelet interaction. Increased levels of sCD40L have reported to be correlated with an increased risk of cardiac events and it is expressed by activated platelets and increased plasma levels have been associated with platelet activation.[91]

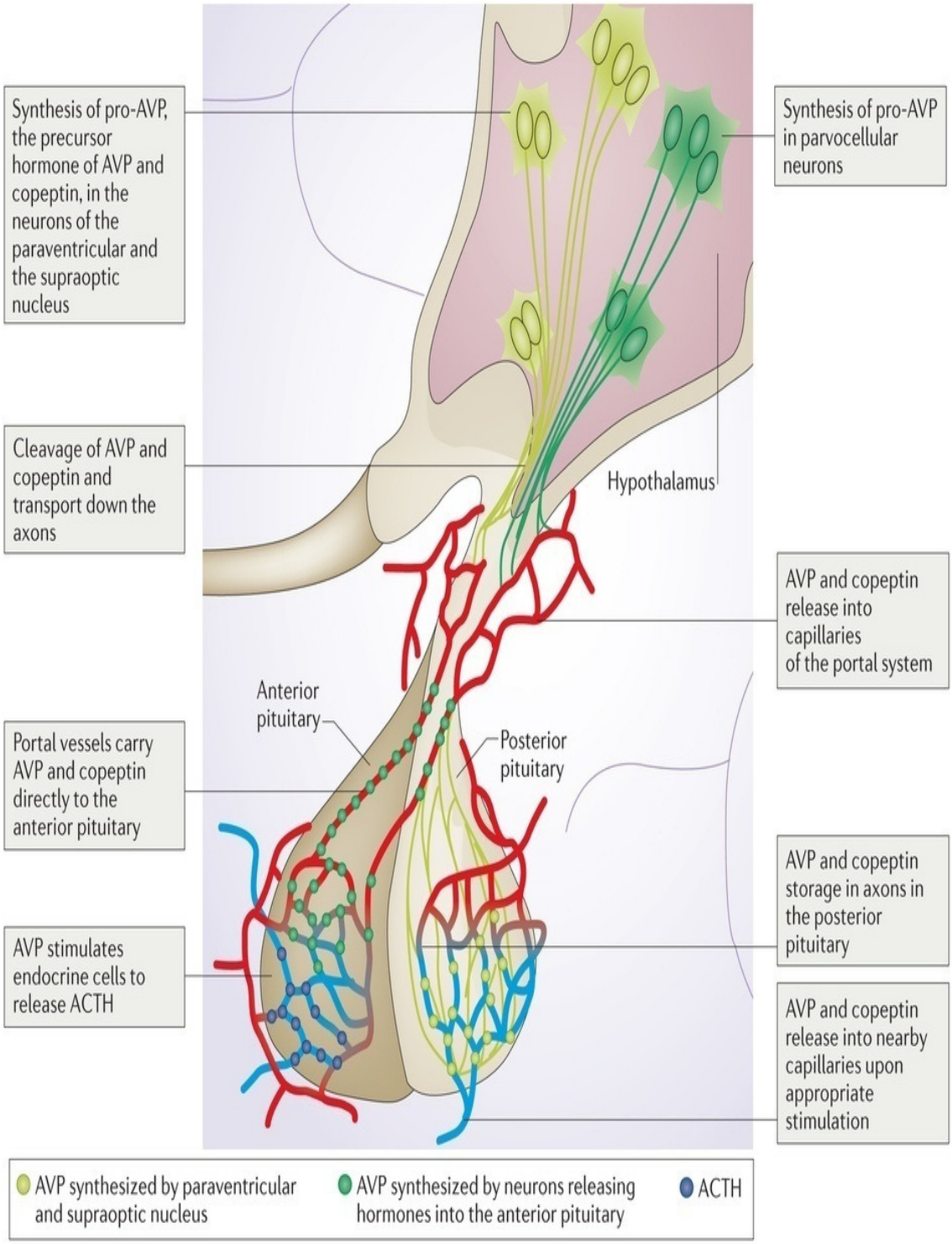
ISCHEMIA MODIFIED ALBUMIN (IMA):

Current cardiac biomarkers are markers of cell death which occurs in AMI. A novel marker, IMA is secreted when the circulating serum albumin contacts ischemic heart tissues. IMA can be estimated by Albumin Cobalt Binding (ACB) assay which is based on the IMA's ability to bind to cobalt.[81]

PREGNANCY ASSOCIATED PLASMA PROTEIN-A:

PAPP-A is a high molecular weight glycoprotein synthesized by the syncytiotrophoblast ,a zinc binding metalloproteinase classified under matrix metalloproteinase (MMP). Increased levels of PAPP-A were found in the patients with the unstable plaque. It is usually estimated during pregnancy in case of screening for Down's Syndrome. Human fibroblasts contains PAPP-A and released into circulation during the disruption of atherosclerotic plaque. Bayes-Genes et all demonstrated an increased PAPP-A concentrations in serum of patients with the unstable angina and acute coronary syndromes. PAPP-A has also been evaluated as a marker of cardiovascular risk in symptomatic hyperlipidemic individuals showing an association with degree of echogenicity of carotid atherosclerotic plaques.In a preliminary study, PAPP-A showed patterns are highly variable release ranging from 2 to 30 hours after the onset of symptom.[92]

SYNTHESIS OF COPEPTIN



HEART FATTY ACID BINDING PROTEIN:

H-FABP has a molecular weight of 14-15 kiloDalton which is very stable. It is made of 132 aminoacids. It is a soluble cytoplasmic protein of myocardial cells. It is involved in the transport and metabolism of fatty acid and expressed mainly in the myocardium. However,brain, kidney and skeletal muscle also have shown its minimal expression. Small size and its water solubility facilitates its rapid diffusion through the interstitial space and it appears as early as 90 minutes after symptoms of onset and peak is attained within 6 hours due to rapid renal clearance.[92]

ARGININE VASOPRESSIN

Arginine vasopressin (AVP) is also called as anti diuretic hormone, which is one of the main hormones of the hypothalamic -pituitary-adrenal (HPA) axis. The functions of AVP are water regulation and homeostasis of electrolytes. The main stimuli for AVP release is hyperosmolarity. Hypoxia,hypotension,acidosis,pain, infection, insulin-induced hypoglycaemia,certain drugs, nausea, vomiting, and non-specific causes of stress can also rise the concentration of AVP in circulation.

COPEPTIN

COPEPTIN is a glycopeptide with 39 -aminoacids and it has a leucine-rich core segment. It is a C-terminal part of pro-AVP (CT-proAVP),which was first explained by Holwerda in 1972. Thus, AVP and COPEPTIN share the common

precursor peptide, the preprovasopressin, which consists of a signal peptide, AVP, neurophysin II, and COPEPTIN. These substances are separated during the axon transport from the cell body to the axon terminals in the gland of posterior pituitary.

Hence,COPEPTIN is stored in the neurohypophyseal vesicles along with AVP and neurophysin II and is secreted in equimolar amounts with AVP in the blood circulation.[5,8,93] As AVP, COPEPTIN is also released in circulation during stress.

METHODS OF MEASUREMENT:

ELISA

IMMUNOLUMINOMETRIC ASSAY

AIM AND OBJECTIVES

AIM:

To estimate the serum level of COPEPTIN in patients with Acute Myocardial Infarction within four hours of onset of chest pain.

OBJECTIVES:

1. To correlate the S.COPEPTIN level with S.CK-MB.
2. To evaluate the correlation between S.COPEPTIN and other several known risk factors for AMI such as Random Blood Sugar, Blood.Urea, S.Creatinine and Lipid profile (S.Total Cholesterol, S.Triglycerides, S.HDL-C, S.LDL-C, S.VLDL).
3. To prove the use of S.COPEPTIN as an early marker of AMI.

MATERIALS AND METHODS

The study was conducted in Thanjavur Medical College, Thanjavur. 50 patients with symptoms of Acute Myocardial Infarction presented within 4 hrs of onset of pain in the casuality with ECG findings correlated and were taken as subjects.50 age and sex matched controls were taken as control group.

INCLUSION CRITERIA:

1) Patients with complaints of chest pain within 4hrs of onset.

2) Electro cardiac graphic findings showing abnormal ST-T wave changes (ST segment elevation or depression or deep symmetrical T wave inversion)

EXCLUSION CRITERIA:

1) Presence of renal diseases.

2) Presence of cirrhosis.

3) Chronic obstructive pulmonary disease.

4) Presence of stroke, penetrating chest wounds, skeletal muscle injury, malignancy, trauma.

5) Critically ill patients.

6) Any infectious diseases.

SAMPLE COLLECTION:

Informed consent was obtained for each patient and control prior to the study.Under strict aseptic precautions, venous blood samples were drawn as soon as the subjects got admitted as per the inclusion criteria. The samples are allowed to clot for 30 minutes and were centrifuged at 3000g for 10 minutes. The sera for estimating S.COPEPTIN were stored in the deep freezer, until the estimation was done.

The following parameters were estimated immediately after the serum separation.

1. Serum COPEPTIN
2. Serum Creatine Kinase -MB
3. Random Blood Sugar
4. Blood Urea
5. Serum Creatinine
6. S.Total Cholesterol
7. S.Triglycerides.
8. S.HDL cholesterol

CALCULATED PARAMETERS :

1. Body Mass Index:(BMI) = Weight in Kg/(Height in meters)

2. S.Very Low Density Lipoprotein =TGL/5

3.S.Low Density Lipoprotein =T.Chol – (LDL +VLDL)

ESTIMATION OF SERUM COPEPTIN :

Serum COPEPTIN was measured in all the samples within one month of collecting the samples by Sandwich Enzyme – Linked Immuno Sorbent Assay(ELISA)

PRINCIPLE

The basis of ELISA,used by this kit is Biotin double antibody sandwich technology to assay the levels of Human COPEPTIN (CPP).COPEPTIN (CPP) is added to each wells, which are pre-coated with Copeptin (CPP) monoclonal antibody and then,it was incubated. Then, anti CPP antibodies labelled with biotin is added to unite with streptavidin-HRP, which forms the immune complex.

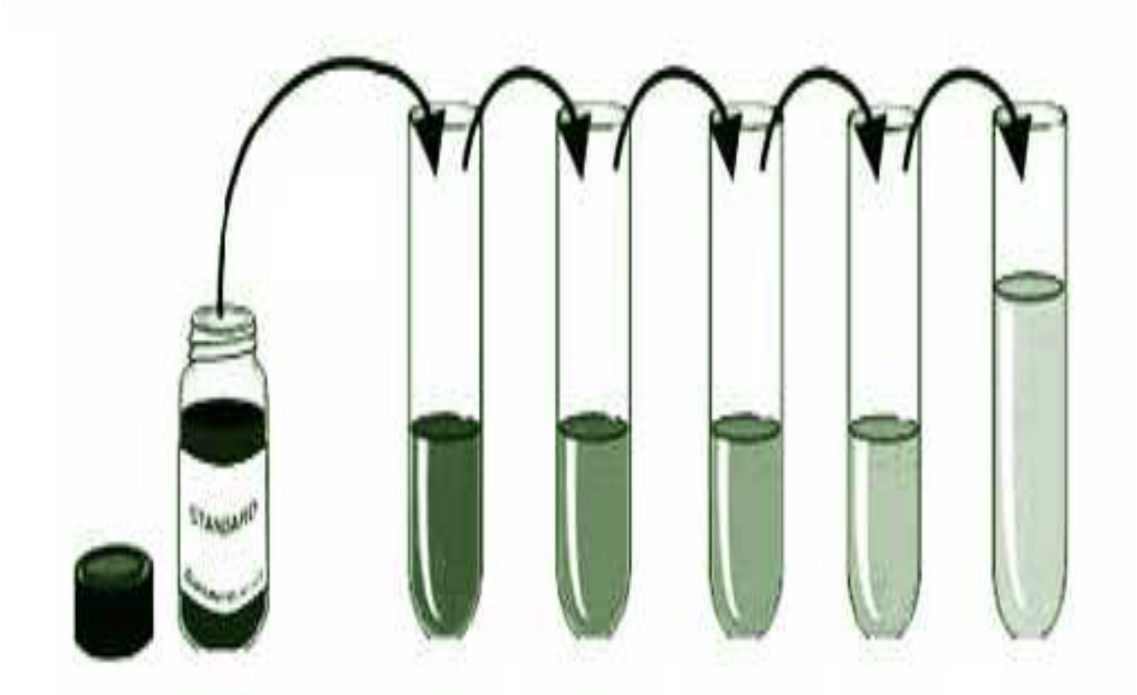
The unbound enzymes are removed after incubation period and the kit is washed. Then substrate A and B is added. The solution will turn blue and change into yellow colour with the effect of acid. The shades of solution and the concentration of Human COPEPTIN (CPP) are positively correlated.

REAGENTS PROVIDED IN THE KIT :

1	Standard solution (1600 ng/L)	0.5 ml	7	Chromogen Solution A	6 ml
2	Standard dilution	3 ml	8	Chromogen Solution B	6 ml
3	Coated ELISA plate	12 well 8 tubes	9	Stop solution	6 ml
4	Streptavidin-HRP	6 ml	10	Instruction	1
5	Washing concentrate(30X)	20 ml	11	Sealplate membrane	2
6	Anti CPP antibodies labeled with biotin	1 ml	12	Hemetic bag	1

PROCEDURE:

1. **DILUTION OF THE STANDARD SOLUTIONS:** This kit has a standard of original concentration,which could be diluted in small test tubes .



1600ng/L 800ng/L 400ng/L 200ng/L 100ng/L 50ng/L

2.The number of stripes needed was determined by that of samples to be tested added by that of given standards. Then, each standard solution and blank well should be arranged with three or more wells as much as possible.

3.SAMPLE INJECTION:

A) Blank well: No sample, anti CPP antibody labeled with biotin or streptavidin-HRP was added to comparison blank well except chromogen solutions A & B and stop solution while taking same steps that follow.

800ng/L	Standard No.5	Original Standard + 120µl Standard diluents
400ng/L	Standard No.4	Standard No.5 + 120µl Standard diluents
200ng/L	Standard No.3	Standard No.4 + 120µl Standard diluent
100ng/L	Standard No.2	Standard No.3 + 120µl Standard diluent
50ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

B) Standard solution well: 50µl of standard and 50µl of streptavidin-HRP were added.(Biotin antibodies have united in an advance in the standard.Therefore,no biotin antibodies are added.)

C) Sample well : 40µl of sample, 10µl of CPP antibodies, 50µl of streptavidin-HRP were added & then covered with the seal plate membrane and mixed them & incubated at 37℃ for 60 minutes.

4. PREPARATION OF WASH SOLUTION: The washing concentration (30X) was diluted with the distilled water for later use.

5. WASHING: The seal plate membrane was removed. The liquid was removed. Each well was filled with washing solution. After 25 seconds, the liquid was drained. Then this washing procedure was repeated five times and this plate was blotted.

6. COLOR DEVELOPMENT: 50μl of chromogen solution A was added firstly to each well and then,50μl of chromogen solution B was added to each well. Mixed & Incubated for 10 minutes at 37℃ away from light for the color development.

7. STOP: 50μl of Stop Solution was added to every wells to stop the reaction (the color blue changes into yellow immediately.)

8. ASSAY OF COPEPTIN: Blank well as zero was taken & absorbance (OD) of each well was measured one by one under the 450nm wavelength, within 10 minutes after adding of the stop solution.

9. CALCULATION OF RESULTS : According to standard's concentration and corresponding OD values,the linear regression equation of standard curve was calculated. Then according to OD value of the given samples, the concentration of corresponding sample was calculated. The Special software could be employed to calculate as well.

ESTIMATION OF SERUM (CK-MB)

METHOD-Modified IFCC method.

PRINCIPLE -

This test involves measurement of CK activity in presence of an antibody to the CK-M monomer. So,this antibody completely inhibits an activity of CK-MM and half of the activity of CK-MB while not affecting B subunit activity of CK-MB and CK-BB. Then this, CK method is used to quantitatively determine the activity of CK-B. The CK-MB activity was calculated by multiplying the CK-B activity by two.

Creatine phosphate +ADP $\xrightarrow{\text{Creatine kinase}}$ Creatine +ATP

ATP +D-Glucose $\xrightarrow{\text{Hexokinase}}$ Glucose-6-phosphate+ATP

Glucose-6-phosphate + $NADP^+$ $\xrightarrow{\text{Glucose 6 Phospate dehydrogenase}}$ 6 - Phospogluconate + NADPH+ H^+

REAGENTS

REAGENT I (Buffer/Enzymes)

REAGENT II (Polyclonal Antibody)

WORKING REAGENT:

4 ml of reagent I was added to one ml of reagent II. Mixed it by gently swirl till completely dissolved.

PROCEDURE:

The reagent and sample are bought to room temperature.

	Blank	Standard	Test
Reagent	1ml	1ml	1ml
Distilled Water	50µL	-	-
Standard	-	50µL	-
Sample	-	-	50µL

To 1 ml of working reagent ,50µL of sample was added and read immediately at 340 nm.

NORMAL VALUES:

Serum:0-24 U/L

ESTIMATION OF GLUCOSE:

Method: Glucose Oxidase – Peroxidase method (End point)

Principle:

Glucose present in the sample is oxidised to Gluconic acid and hydrogen peroxide by the enzyme,Glucose oxidase (GOD). Peroxidase enzyme (POD) acts on hydrogen peroxide to yield water and nascent oxygen. This nascent oxygen oxidizes phenol, which in turn combines with 4-aminoantipyrine to form a coloured quinoneimine complex. The intensity of the colour is directly proportional to the concentration of the sample..

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4\text{AAP} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 2H_2O$$

Glucose standard: 100 mg/dl

Enzyme Reagent Composition:

Glucose oxidase : ≥ 20000 U/L

Peroxidase : ≥ 2000 U/L

Phenol : 10 mmol/L

Phosphate buffer : 200 mmol/L.

Specimen: Fresh unhemolysed serum.

Assay Parameters:

Wavelength-1 : 505 nm

Wavelength-2 : 670 nm

Reaction time : 5 minutes

Reaction temperature : 37ºC

Reagent volume : 1000 µl

Sample volume : 10 µl

Blank Absorbance Limit : 0.2

Units : mg/dl

Procedure:

Pipette into the test tubes labelled as	Blank	Standard	Test
Enzyme reagent	1ml	1ml	1ml
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

The contents of the tubes were mixed well after each addition and incubated at 37ºC for 5 minutes. The absorbance of standard and test was read at 505 nm.

Calculation:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{concentration of Standard}$$

Linearity: Up to 500 mg/dl.

Normal values: Glucose (in fasting): 70 – 110 mg/dl

Glucose (in post prandial): 90 – 140 mg/dl

QUANTITATIVE DETERMINATION OF UREA BY UREASE METHOD (GLUTAMATE DEHYDROGENASE - FIXED TIME)

The method is used to determine urea in serum / plasma.

METHODOLOGY

Itis a kinetic, enzymatic method.

PRINCIPLE OF THE METHOD

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \text{2-Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of change of absorbance at 340nm is directly proportional to the concentration of urea in serum.

REAGENT COMPOSITION

Tris buffer (pH 7.8) : 96 mmol/L

ADP : 0.6 mmol/L

Urease : 16000U/L

GLDH : 960 U/L

NADH : 0.25 mmol/L

2-Oxoglutarate : 9 mmol/L

Urea standard : 50 mg/dl

WORKING REAGENT PREPARATION & STABILITY

Working reagent is prepared by mixing 4 parts of reagent R1 with one part of reagent R2.

The working reagent (4R1 : 1R2) is stable for about 30 days at 2-8°C, when it was protected from light and contamination. Fresh working solution is prepared before the assay is performed.

STORAGE & STABILITY

Reagent solutions R1 and R2 and original standard are stable when unopened, till the expiry date when stored at 2-8°C.

Reagent deterioration may be detected when there is turbidity or when the reagent blank absorbance is <0.8 at 340nm.

ASSAY PARAMETERS

Mode : fixed time

Wavelength : 340 nm

Sample volume : 20 µl

Reagent volume : 1000 µl

Lag time : 20 seconds

Kinetic interval : 60 seconds

No. of readings : 1

Reaction temperature : 37°C

Reaction direction : decreasing

Normal low value : 13 mg/dl

Normal high value : 45 mg/dl

Linearity low value : 0 mg/dl

Linearity high value : 250 mg/dl

Absorbance limit (min.) : 0.8

Blank with : water

Concentration of standard : 50 mg/dl

Units : mg/dl

ASSAY PROCEDURE

PIPETTE	STANDARD	TEST
Working reagent	1000 µl	1000 µl
Standard	20 µl	-
Sample	-	20 µl

CALCULATION

Urea concentration in mg/dl = $\frac{\Delta \text{ Absorbance of the test}}{\Delta \text{ Absorbance of standard}} \times$ concentration of standard(mg/dl)

REFERENCE VALUES

In serum/plasma : 13 - 45 mg/dl

LINEARITY

The assay is linear upto 250 mg/dl. For very higher values the samples were diluted with normal saline and the assay was repeated. Then results were multiplied with the dilution factor.

SENSITIVITY : 2.0 mg/dl

INTERFERENCE:

Haemoglobin interferes upto 400 mg/dl, ascorbate upto 30 mg/dl, bilirubin upto 30 mg/dl and triglycerides upto 2000 mg/dl do not interfere with the test.

ESTIMATION OF SERUM CREATININE

MODIFIED JAFFE'S REACTION, INITIAL RATE

PRINCIPLE OF THE METHOD

Creatinine reacts with the picric acid in an alkaline medium to form an orange-yellow color which is termed as Jaffe's reaction. The initial rate method is introduced to improve the specificity of the test.

The optical density of the orange-yellow color formed is directly proportional to the concentration of creatinine, which is measured photometrically at 500 to 520nm.

COMPOSITIONOFTHEREAGENT

- Reagent Number 1 - Picric Acid Reagent

  Picric acid : 25.8 mmol/L

- Reagent Number 2 – Sodium Hydroxide Reagent

  Sodium hydroxide : 95 mmol/L

- Creatinine standard

  Creatinine standard : 2 mg/dl (0.166 mmol/L)

REAGENT PREPARATION

The equal volumes of Reagent 1 and Reagent 2 are mixed and wait for about 15 minutes before use.

STORAGE AND STABILITY

Reagents 1, 2 and original standard when unopened remain stable till the expiry date. The Working Reagent is stable for about 21 days at 2-8°C. The absorbance of the reagent blank should be <0.3 at 505nm when read against the distilled water.

ASSAY PARAMETERS

Wavelength (nm) : 505

Mode : fixed time

Sample volume : 100 µl

Reagent volume : 1000 µl

Lag time : 20 sec

Kinetic interval : 60 sec

No. of readings : 1

Reaction temperature : 37°C

Normal low : 0.6 mg/dl

Normal high : 1.4 mg/dl

Reaction direction : increasing

Linearity low : 0 mg/dl

Linearity high : 25 mg/dl

Absorbance limit (max) : 0.3

Standard concentration : 2 mg/dl

Blank with : water

Units : mg/dl

ASSAY PROCEDURE

PIPETTE	STANDARD	TEST
Working reagent	1000µl	1000µl
Standard	100µl	-
Test	-	100µl

The test tubes were shaken to mix the contents and the initial absorbance was read within 20 seconds and final absorbance is taken at 80 seconds.

CALCULATION

The results are calculated as follows :

$\Delta A = A_2 - A_1$

$$\text{Creatinine concentration in mg/dl} = \frac{\Delta A \text{ of the test}}{\Delta A \text{ of standard}} \times \text{concentration of standard(mg/dl)}$$

LINEARITY

The assay is linear upto a value of 25mg/dl. For very higher values the samples were diluted with normal saline and this assay repeated. Then the results were multiplied with dilution factor.

NORMALVALUES

For males : 0.7 - 1.4 mg/dl

For females : 0.6 - 1.2 mg/dl

ESTIMATION OF SERUM TOTAL CHOLESTEROL:

Method:

Cholesterol oxidase-Peroxidase Enzymatic, endpoint method.

Principle:

The free cholesterol, liberated from the cholesterol esters by cholesterol esterase (CHE), is oxidized by cholesterol oxidase (CHO) to cholestenone with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine (4AAP) and a phenolic compound in the presence of peroxidase (POD) to yield a red coloured complex.

Cholesterol ester + Water $\xrightarrow{\text{CHE}}$ Cholesterol + Fatty acid

Cholesterol + Oxygen $\xrightarrow{\text{CHO}}$ Cholest-4-en-3-one + H_2O_2

$2H_2O_2$ + 4AAP+ Phenol $\xrightarrow{\text{POD}}$ Quinoneimine dye + $4H_2O$

The absorbance of quinoneimine formed is directly proportional to the cholesterol concentration.

Reagent Composition:

Goods buffer (pH – 6.4) : 100 mmol/L

Cholesterol oxidase : >100 U/L

Cholesterol esterase : >200 U/L

Peroxidase : >3000 U/L

4 – Amino antipyrine : 0.3 mmol/L

Phenol : 5 mmol/L

Cholesterol standard: 200mg/dl

Assay Parameters:

Wavelength-1 : 505 nm

Wavelength-2 : 670 nm

Incubation time : 10 minutes

Incubation temperature : 37ºC

Reagent volume : 1000 µl

Sample volume : 10 µl

Absorbance Limit : 0.4

Blank with : Reagent

Units : mg/dl

Assay procedure: (End Point Method)

Pipette into the test tubes labelled as	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

Mixed well and incubated for 10 min at room temperature. The absorbance of the test and standard were read against the reagent blank at 505 nm.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Reference range: 150-200 mg/dl

Linearity: Up to 1000 mg/dl

Sensitivity: 1mg/dl

Interference: Hemoglobin upto 200mg/dl, Ascorbic acid upto 12mg/dl, Bilirubin upto 10mg/dl and Triglycerides upto 700 mg/dl do not interfere with the test.

ESTIMATION OF SERUM TRIGLYCERIDES:

Method: GPO-PAP method, Endpoint.

Methodology: Colorimetric, Enzymatic method with Glycerol Phosphate Oxidase (GPO).

Principle: Lipoprotein lipase (LPL) catalyzed hydrolysis of Triacylglycerol, yields Glycerol which is phosphorylated by Glycerol kinase (GK) using ATP to Glycerol-3-phosphate, which upon oxidation by the Glycerol Phosphate Oxidase (GPO), yields Di-Hydroxy Acetone Phosphate and Hydrogen peroxide. The Hydrogen peroxide reacts with Phenol and 4-Amino Anti Pyrine in presence of Peroxidase

(POD) to form a coloured complex. The intensity of Quinoneimine dye formed is proportional to the Triglyceride concentration in the sample.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol 3 phosphate} + \text{ADP}$$

$$\text{Glycerol 3 phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$H_2O_2 + \text{4AAP} + \text{3,5-DHBS} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + 2H_2O$$

[DHAP -Di-Hydroxy Acetone Phosphate

ATP - Adenosine Tri Phosphate

4-AAP - 4 Amino Anti Pyrine

DHBS -3,5 Dichloro-2 Hydroxy Benzene Sulfonate]

Triglycerides standard concentration- 200mg/dl

Reagent composition:

Buffer (pH – 7.0) : 40 mmol/L

4-AAP : 0.4 mmol/L

ATP : 2.0 mmol/L

Mg^{2+} : 2.5 mmol/L

DHBS : 0.2 mmol/L

Glycerol kinase : 1500 U/L

Glycerol 3 – phosphate oxidase : 4000 U/L

Peroxidase : 2200 U/L

Lipoprotein lipase : 4000 U/L

Reagent Preparation:

Reagent 1 (R1) - Enzymes / chromogen

Reagent 2 (R2) - Buffer

The working reagent was prepared by mixing 4 parts of Reagent 1 with 1 part of Reagent 2.

Sample: Unhemolysed serum collected after 12 hrs of fasting.

Assay Parameters:

Wavelength-1 : 505 nm

Wavelength-2 : 670 nm

Incubation time : 10 minutes

Incubation temperature : 37ºC

Reagent volume : 1000 µl

Sample volume : 10 µl

Absorbance Limit : 0.5

Blank with : Reagent

Units : mg/dl

Assay Procedure:

Pipette into test tubes labelled as	**Blank**	**Standard**	**Test**
Working reagent	1000µl	1000µl	1000µl
Distilled water	10µl	-	-
Standard	-	10µl	-
Sample	-	-	10µl

Mixed and incubate for 10min, at room temperature. Absorbance was read at 505nm for standard and sample against reagent blank.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Reference values: 50 -150 mg/dl

Linearity: Upto 1000mg/dl

Sensitivity: 2mg/dl

Interferences: Hemoglobin upto 300mg/dl, Ascorbate upto 3mg/dl and Bilirubin level upto 20mg/dl does not interfere with the test.

ESTIMATION OF SERUM HDL - CHOLESTEROL:

METHOD: Phosphotungstic acid method, Endpoint.

PRINCIPLE:LDL,Chylomicrons (CM) and VLDL are precipitated from serum or plasma with Phosphotungstate in presence of divalent cations such as Magnesium. The HDL cholesterol remains unaffected in the supernatant fluid and is estimated using the cholesterol reagent.

$$\text{Serum/plasma} \xrightarrow{\text{Phosphotungstic acid, } Mg^{2+}} \underset{\text{(supernatant)}}{\text{HDL}} + \underset{\text{(precipitate)}}{\text{[LDL+VLDL+CM]}}$$

REAGENT COMPOSITION: Precipitating reagent:

Phosphotungstic acid	0.4 mmol/L
Magnesium chloride	20 mmol/L

HDL cholesterol standard – 15mg/dl

Sample: Unhemolysed serum

PRECIPITATION:

Precipitation of LDL, VLDL and Chylomicrons done as follows:

Pipette into tubes	Volume
Sample	200µl
Precipitating reagent	500µl

Mixed well and this reaction mixture was allowed to stand for about 10 min at room temperature, centrifuged at 4000 rpm for 10min and the clear supernatant fluid was collected. The supernatant fluid was used to estimate the concentration of HDL cholesterol in the sample.

Assay procedure: Mixed well and incubated for 10 minutes at room temperature.

Pipette into test tubes marked	Blank	Standard	Test
Cholesterol working reagent	1000µl	1000µl	1000µl
Distilled water	100µl	-	-
HDL Cholesterol standard	-	100µl	-
Sample Supernatant	-	-	100µl

The absorbance of the standard and the test samples were read at 500 nm against reagent blank.

Calculation:

$$\textbf{HDL Cholesterol (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X Conc. of std. X Dilution factor}$$

$$= \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } 52.5$$

= mg/dl HDL Cholesterol.

Reference values: 35 – 60 mg/dl

Linearity: 150 mg/dl; **Detection limit**: 3.0 mg/dl

ESTIMATION OF SERUM LDL CHOLESTEROL:

BY FRIEDEWALD FORMULA:

LDL = TOTAL CHOLESTEROL – (HDL + VLDL)

VLDL = TGL/5, if TGL is less than 400mg/dl.

Reference values: Serum / plasma LDL: 100 – 129 mg/dl

VLDL: < 40 mg/dl.

MASTER CHART-I CONTROL GROUP

S.NO	Age (Yrs)	Sex	Wt (Kg)	Ht (mts)	BMI (kg/m2)	SBP (mm Hg)	DBP (mm Hg)	C0PEPTIN (ng/L)	CKMB (U/L)	RBS (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)	T-CHOL (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
1	54	M	64	1.6	24	122	82	33	5	96	15	0.6	150	112	45	22	83
2	51	M	50	1.5	22	110	70	23	6	93	13	0.7	176	108	43	22	111
3	54	M	70	1.65	25	100	80	21	7	91	16	0.68	198	112	52	22	124
4	61	M	60	1.53	25	120	80	10	8	90	17	0.78	198	113	50	23	125
5	58	M	65	1.65	23	110	80	13	9	98	18	0.65	199	132	48	27	124
6	54	M	55	1.48	25	100	82	12	10	92	21	0.69	178	111	48	22	108
7	49	M	60	1.48	27	122	70	21	11	98	22	0.65	197	123	49	25	123
8	57	M	63	1.45	29	123	72	23	6	96	23	0.61	197	124	38	25	134
9	48	M	60	1.55	24	130	76	23	11	89	24	0.72	199	123	39	25	135
10	49	M	62	1.54	26	100	80	23	5	90	31	0.63	199	112	48	22	129
11	61	M	55	1.5	24	120	80	23	10	92	32	0.65	196	145	38	29	129
12	60	M	58	1.52	25	114	74	12	15	94	31	0.75	156	134	38	27	91
13	54	M	65	1.55	27	110	70	32	6	98	32	0.67	156	125	39	25	92
14	58	M	55	1.6	21	130	80	23	5	86	34	0.86	156	126	40	25	91
15	52	M	60	1.52	25	110	80	32	6	92	27	0.59	156	124	40	25	91
16	53	M	60	1.53	25	126	84	27	4	96	27	0.59	156	125	38	25	93
17	54	M	58	1.52	25	124	80	30	4	90	23	0.58	156	112	39	22	95
18	58	M	55	1.48	25	110	80	30	5	96	26	0.87	176	134	40	27	109
19	54	M	55	1.6	21	128	80	32	5	88	27	0.59	185	134	40	27	118
20	49	M	72	1.67	25	122	80	34	6	98	28	0.68	192	142	41	29	122
21	51	M	65	1.65	23	126	82	34	4	86	29	0.67	191	132	41	27	123
22	52	M	60	1.67	21	120	80	30	4	84	32	0.69	191	132	37	27	127
23	57	M	70	1.7	24	112	74	30	9	90	18	0.7	190	132	29	27	134
24	55	M	75	1.6	29	130	80	32	15	80	23	0.7	190	123	32	25	133
25	54	M	70	1.6	27	120	80	32	15	98	14	0.8	189	123	32	25	132
26	49	M	70	1.68	24	128	74	23	6	90	18	0.8	189	132	41	27	121
27	45	M	80	1.7	27	110	70	11	13	102	16	0.7	188	123	41	25	122
28	51	M	69	1.65	25	120	80	44	6	104	23	0.7	188	134	41	27	120
29	48	M	75	1.68	26	116	84	38	16	98	34	0.8	187	143	41	29	117
30	52	M	75	1.72	25	110	72	29	9	84	26	0.7	187	143	41	29	117
31	43	M	72	1.69	25	110	70	39	18	96	26	0.8	186	123	32	25	129

32	54	M	70	1.65	25	122	82	50	19	102	27	0.8	154	123	32	25	97
33	52	M	69	1.6	26	110	80	43	14	92	27	0.7	167	123	32	25	128
34	51	M	70	1.67	25	126	80	50	11	82	32	0.78	185	123	32	25	128
35	58	M	75	1.71	25	120	82	32	11	106	32	0.67	184	143	32	29	123
36	46	M	64	1.6	24	126	84	46	11	106	32	0.56	184	143	32	29	123
37	49	M	74	1.68	26	110	80	46	11	104	26	0.56	183	148	32	30	121
38	43	M	70	1.65	25	114	78	52	11	102	28	0.66	183	149	32	30	121
39	45	M	75	1.68	26	130	80	54	11	86	28	0.66	182	145	32	29	121
40	48	F	74	1.69	25	110	70	50	9	90	29	0.77	182	145	42	29	111
41	52	F	67	1.67	24	116	84	54	10	104	26	0.78	181	149	36	30	115
42	46	F	70	1.68	24	120	80	54	11	84	26	0.78	180	149	43	30	107
43	49	F	67	1.67	24	110	70	58	12	98	27	0.78	154	146	34	29	91
44	47	F	71	1.7	24	120	80	52	15	102	27	0.78	179	145	34	29	116
45	45	F	72	1.67	25	120	80	49	14	82	25	0.78	154	149	34	30	90
46	52	F	80	1.7	27	126	82	45	11	102	24	0.78	178	148	34	30	114
47	51	F	65	1.72	21	120	80	54	11	92	26	0.78	178	145	34	29	115
48	54	F	72	1.69	25	118	76	32	11	104	27	0.78	177	146	34	29	114
49	51	F	68	1.62	25	120	80	50	11	98	25	0.8	177	145	34	29	114
50	56	F	74	1.68	26	110	70	50	11	86	14	0.8	176	145	43	29	104

MASTER CHART – II STUDY GROUP

S. No	Age (yrs)	Sex	Wt (kg)	Ht (mts)	BMI (kg/m2)	SBP (mm /Hg)	DBP (mm / Hg)	Duration (hours)	COPEPTIN (U/L)	CKMB (U/L)	RBS (mg/ dl	UREA (mg/ dl)	CREAT (mg/dl)	T-CHOL (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl
1	46	M	55	1.5	24	100	70	3	80	11	86	19	0.67	211	132	31	26	154
2	49	M	64	1.56	26	150	100	4	32	13	98	25	0.56	213	143	32	29	152
3	47	M	60	1.55	24	110	80	4	159	34	108	24	0.59	214	145	37	29	148
4	45	M	65	1.52	28	100	80	4	30	8	118	25	0.64	189	154	34	31	124
5	52	M	56	1.55	23	110	70	3	30	8	98	24	0.89	198	156	38	31	129
6	51	M	60	1.6	23	140	100	4	64	8	220	27	0.78	178	167	41	33	104
7	54	M	79	1.6	30	120	80	4	158	24	104	32	0.89	188	167	32	33	123
8	51	M	73	1.65	26	100	80	4	180	34	98	34	0.78	179	165	34	33	112
9	56	M	74	1.72	25	160	100	4	74	29	210	35	0.82	234	176	35	35	164
10	54	M	70	1.68	24	110	70	3	63	30	96	37	0.87	245	156	36	31	178
11	58	M	78	1.64	29	170	110	3	34	21	90	34	0.89	278	176	37	35	206
12	52	M	78	1.67	27	90	70	3	66	20	96	38	0.78	267	167	38	33	196
13	53	M	71	1.7	24	100	80	2	32	11	114	39	0.87	222	158	39	32	151
14	54	M	68	1.6	26	110	70	1	149	28	92	27	0.88	234	189	41	38	155
15	58	M	64	1.68	22	100	80	2	66	25	108	34	0.87	211	178	32	36	143
16	54	M	69	1.7	23	140	90	4	23	26	98	39	0.86	187	178	32	36	119
17	49	M	70	1.65	25	100	80	2	141	34	84	39	0.86	149	167	31	33	85
18	51	M	70	1.62	26	170	110	4	62	28	176	39	0.88	254	168	32	34	188
19	52	M	78	1.6	30	110	80	3	25	29	94	39	0.87	236	187	32	38	166
20	46	M	56	1.8	17	142	90	1	23	30	112	32	0.68	222	154	31	31	160
21	49	M	68	1.8	20	142	90	4	145	36	92	32	0.86	198	154	40	31	127
22	43	M	72	1.64	26	142	80	4	23	11	104	33	0.88	154	112	41	22	91
23	45	M	83	1.67	29	142	80	3	32	24	210	39	0.88	254	136	42	27	185
24	48	M	75	1.78	23	100	70	4	166	24	108	12	0.76	245	165	44	33	168
25	52	M	70	1.78	22	152	94	4	164	20	98	23	0.76	234	165	31	33	170
26	46	M	86	1.64	31	180	110	3	82	18	88	34	0.67	156	164	34	33	89
27	52	M	82	1.7	28	120	80	4	80	28	92	34	0.66	149	165	34	33	82

28	53	M	70	1.71	23	110	70	3	70	26	86	34	0.78	154	154	35	31	88
29	54	M	72	1.6	28	100	80	1	72	32	94	34	0.9	213	137	36	27	150
30	58	M	78	1.7	26	110	90	3	36	8	78	34	0.98	211	112	36	22	153
31	54	M	78	1.67	27	120	80	4	34	6	96	34	0.98	211	111	37	22	152
32	49	M	76	1.72	25	144	96	4	32	6	102	34	0.99	176	158	36	32	108
33	51	M	73	1.7	25	110	80	3	26	7	188	34	0.98	178	112	36	22	120
34	52	M	70	1.7	24	120	80	3	64	11	90	34	0.97	189	187	36	37	116
35	51	M	64	1.87	18	134	70	2	64	9	120	34	0.97	189	156	36	31	122
36	52	M	70	1.7	24	132	80	1	24	11	118	34	0.97	159	123	36	25	98
37	57	M	65	1.6	25	110	80	3	23	6	92	21	0.9	156	145	32	29	95
38	55	M	70	1.65	25	100	70	4	26	6	104	25	0.97	187	123	31	25	131
39	54	M	70	1.7	24	120	70	4	21	5	112	32	0.9	149	143	31	29	89
40	49	F	70	1.64	26	100	70	2	23	4	78	18	0.99	153	124	31	25	97
41	45	F	76	1.72	25	156	80	1	63	8	192	19	0.7	200	111	32	22	146
42	51	F	78	1.7	26	156	80	2	32	5	94	17	0.6	278	167	32	33	213
43	48	F	75	1.68	26	176	90	3	12	5	108	18	0.6	245	143	31	29	185
44	52	F	70	1.64	26	168	100	4	63	7	118	18	0.6	211	111	30	22	159
45	43	F	60	1.72	20	120	80	3	64	5	116	19	0.6	167	112	34	22	111
46	49	F	65	1.6	25	168	90	4	65	5	96	38	0.6	189	123	34	25	130
47	57	F	80	1.69	28	156	88	3	33	5	88	34	0.6	198	123	34	25	139
48	48	F	82	1.7	28	158	80	2	22	5	76	31	0.98	187	123	35	25	127
49	49	F	79	1.72	26	168	90	3	32	5	92	31	0.6	187	132	32	27	125
50	61	F	78	1.64	29	156	90	3	40	5	104	31	0.6	187	112	33	22	132

TABLE 1-Descriptive Statistics of controls and cases

Variables	Control (n=50)				Cases (n=50)			
	Min	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**
AGE	43	61	51.88	4.5	43	61	51.18	4.08
WT	50	80	66.6	7.19	55	86	71.26	7.3
HT	1.45	1.72	1.61	0.08	1.5	1.87	1.67	0.07
BMI	21	29	24.82	1.73	17	31	25.2	2.87
SBP	100	130	118	8	90	180	130	26.03
DBP	70	84	78	4.46	70	110	84	11.14
COPEPTIN	10	58	34.8	13.4	12	180	62.5	46.44
CK-MB	4	19	9.68	3.86	4	36	16.08	10.71
RBS	80	106	93.94	6.97	76	220	110.7	35.14
UREA	13	34	25.08	5.58	12	39	30.14	7.2
CREAT	0.56	0.87	0.71	0.08	0.56	1	0.80	0.14
TC	150	199	179.8	14.5	149	278	201.5	34.9
TGL	108	149	132.4	12.6	111	189	147.7	23.6
HDL	29	52	38.4	5.7	30	44	34.7	3.4
VLDL	22	30	26.7	2.6	22	38	29.6	4.8
LDL	83	135	115	14	82	213	137	33.4

TABLE 2 - Comparison of S.COPEPTIN among cases and controls.

T-TEST			
S.COPEPTIN (ng/L)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	34.8	13.4	p value = .0001 <0.05 – Significant
Cases (n=50)	62.5	46.44	

BAR CHART 1

COMPARISON OF SERUM COPEPTIN LEVEL BETWEEN CONTROLS AND CASES

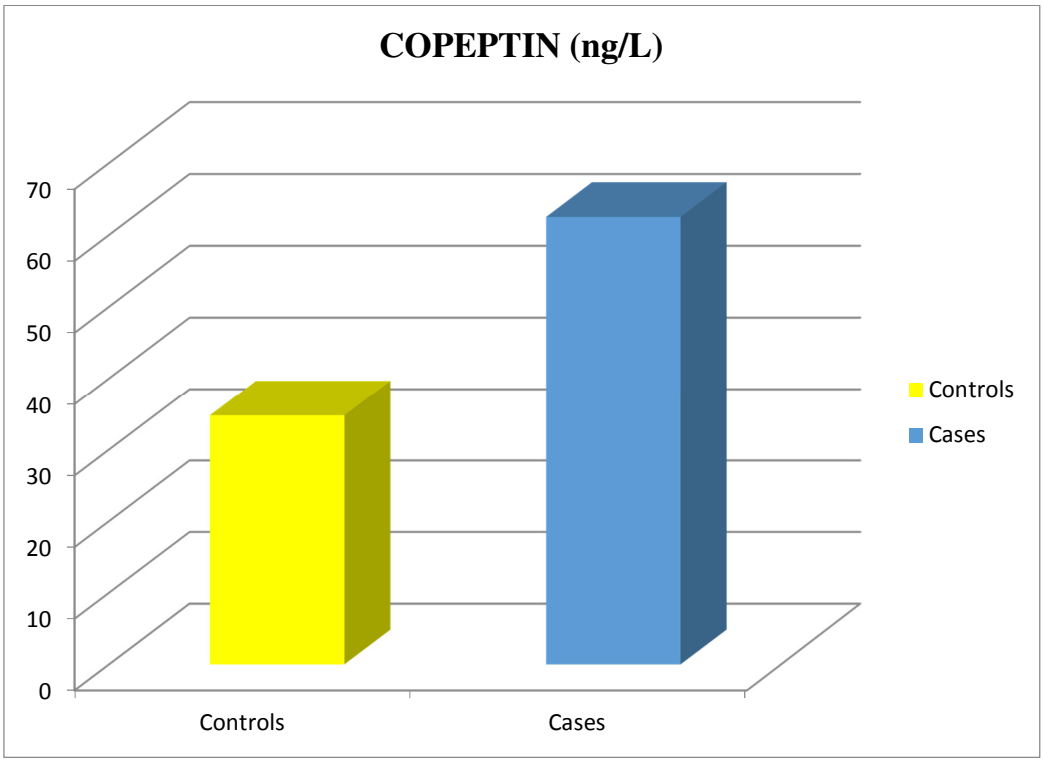


TABLE 3 - Comparison of S.CK-MB among cases and controls.

T-TEST			
S.CK-MB (U/L)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	9.68	3.86	p value = .0001 <0.05 – Significant
Cases (n=50)	16.08	10.71	

BAR CHART 2

COMPARISON OF SERUM CK-MB LEVEL BETWEEN CONTROLS AND CASES

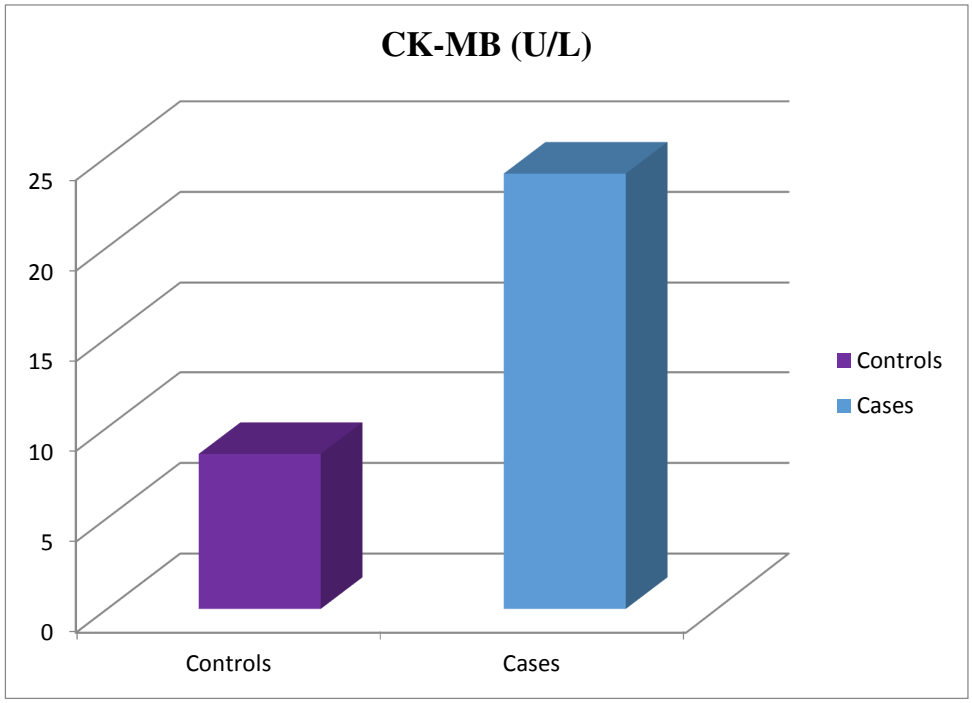


TABLE:4

Shows Scatter diagram represents the correlation between Serum COPEPTIN and time duration in study group.

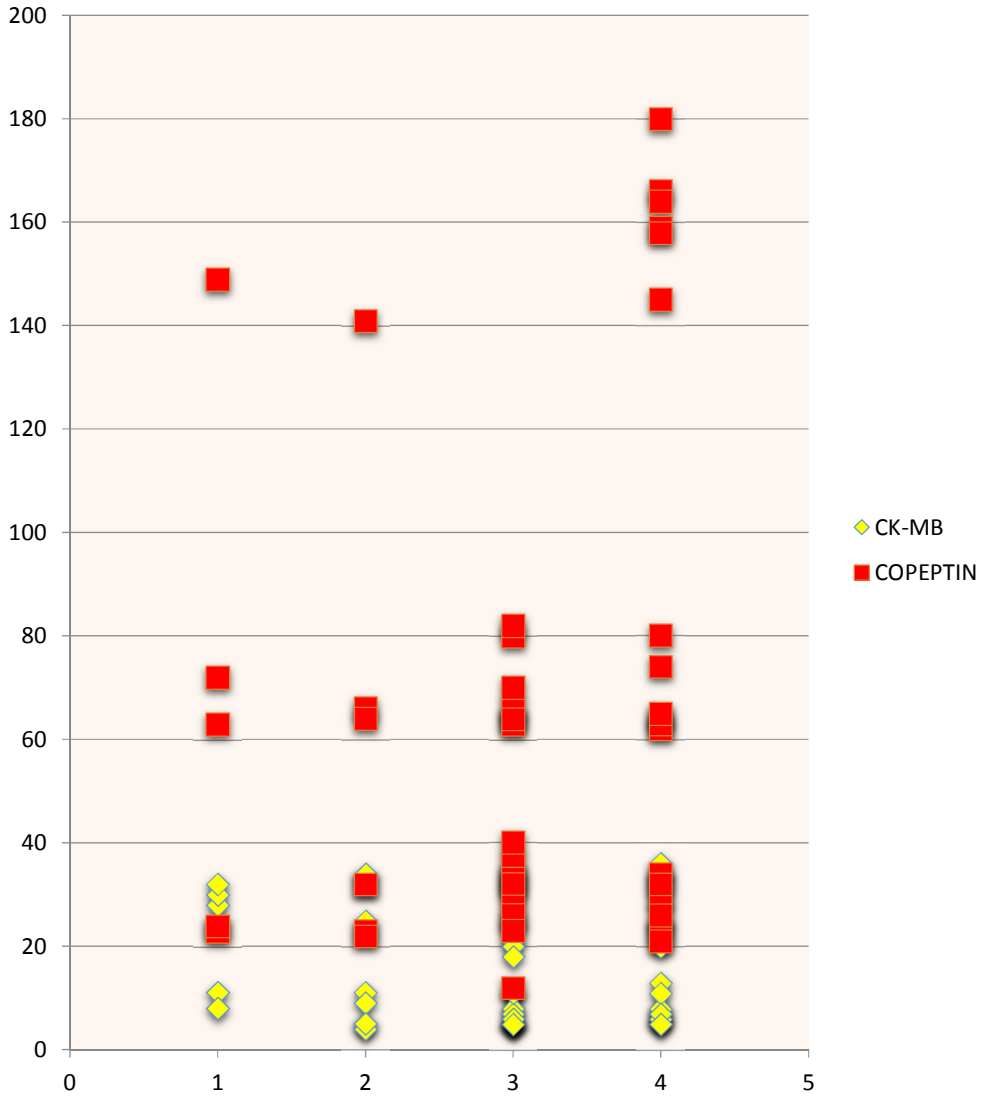


TABLE 5 - Comparison of RBS among cases and controls.

T-TEST			
RBS (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	93.94	6.97	p value = .001 <0.05 – Significant
Cases (n=50)	110.7	35.14	

BAR CHART 3

COMPARISON OF RBS LEVEL BETWEEN CONTROLS AND CASES

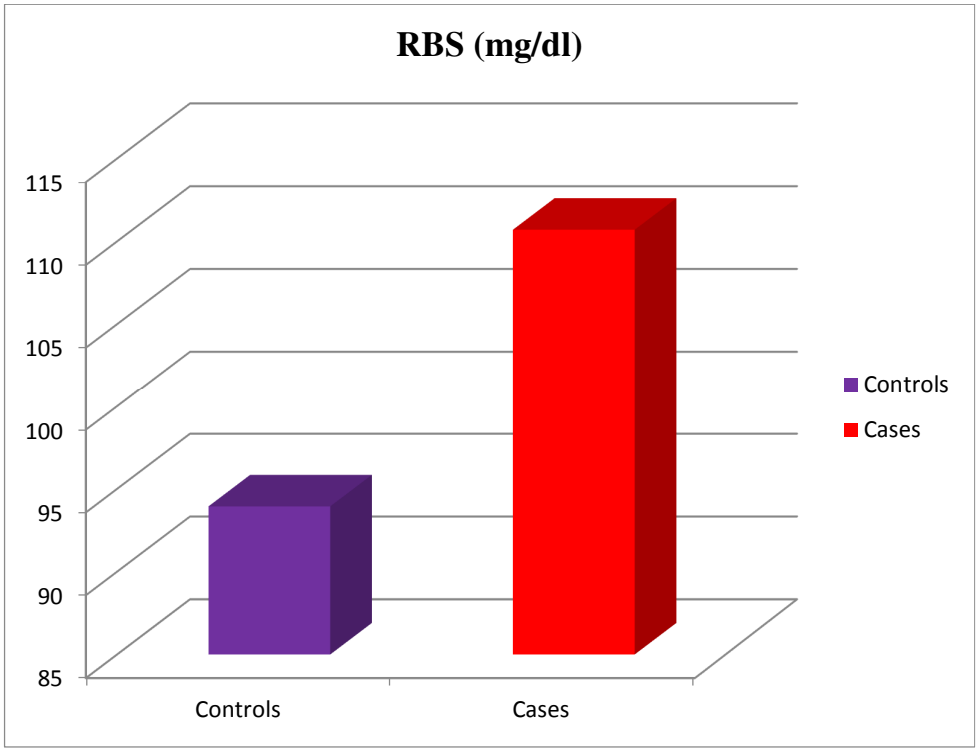


TABLE 6 - Comparison of B.UREA among cases and controls.

T-TEST			
B.UREA (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	25.08	5.58	p value = .0001 <0.05 – Significant
Cases (n=50)	30.14	7.2	

BAR CHART 4

COMPARISON OF B.UREA LEVEL BETWEEN CONTROLS AND CASES

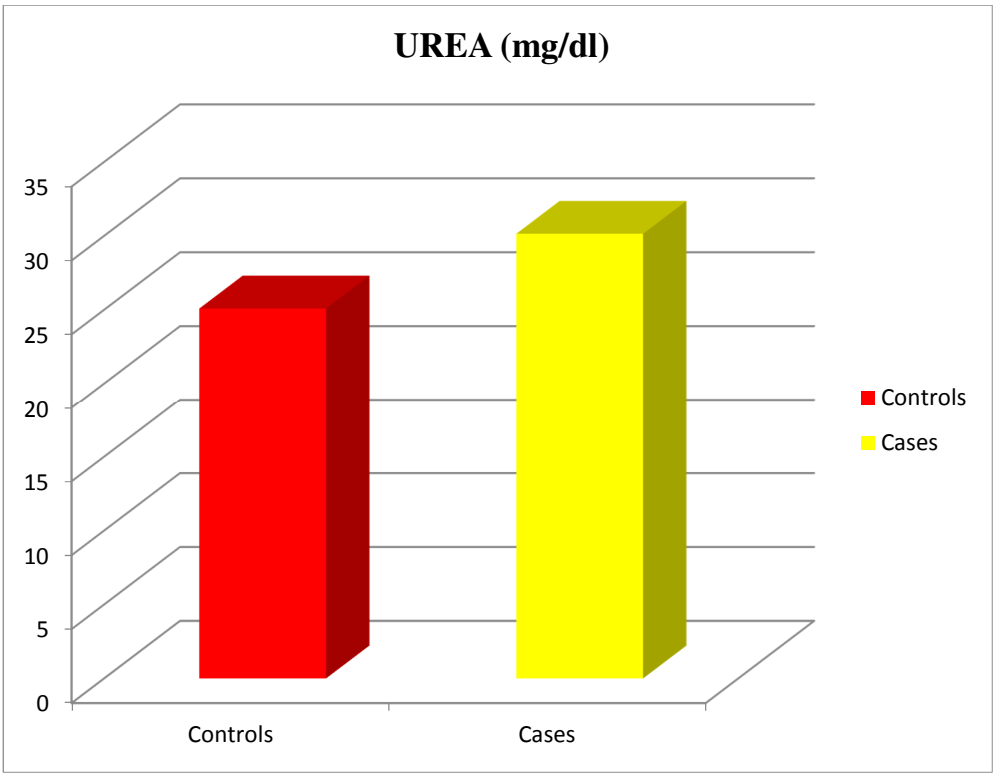


TABLE 7 - Comparison of S.CREATININE among cases and controls.

T-TEST			
S.CREATININE (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	0.71	0.08	p value = .0001 <0.05 – Significant
Cases (n=50)	0.80	0.14	

BAR CHART 5

COMPARISON OF B.UREA LEVEL BETWEEN CONTROLS AND CASES

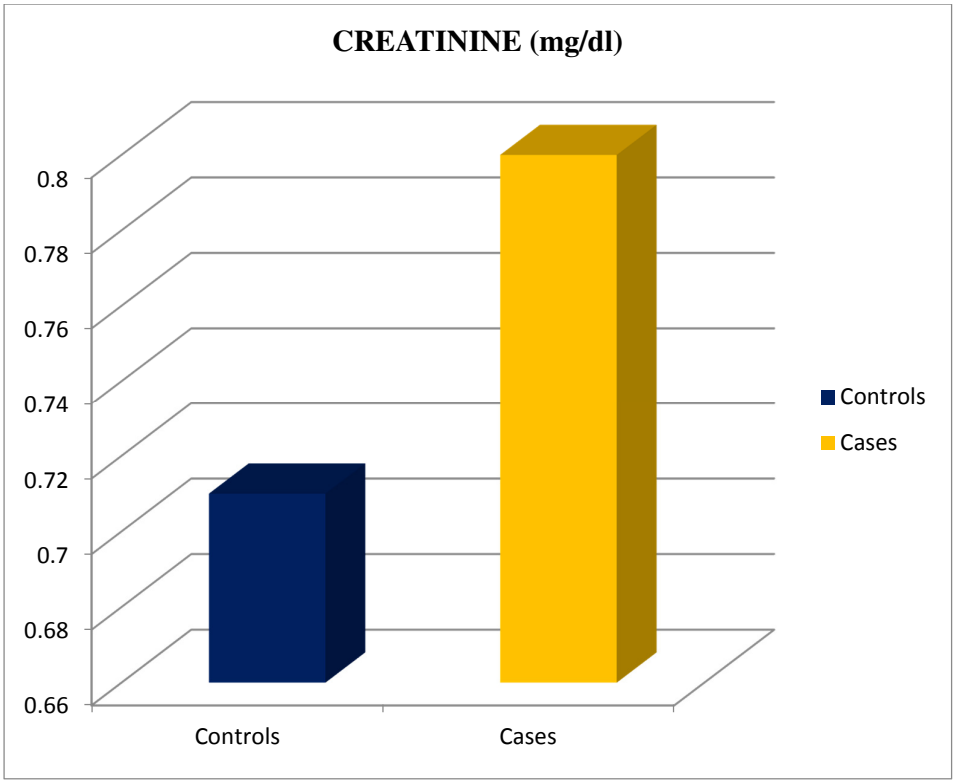


TABLE 8 - Comparison of S.TOTAL CHOLESTEROL among cases and controls.

T-TEST			
S.T.CHOLESTEROL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	179.8	14.5	p value = .0001 <0.05 – Significant
Cases (n=50)	201.5	34.9	

BAR CHART 6

COMPARISON OF SERUM T.CHOLESTEROL LEVEL BETWEEN CONTROLS AND CASES

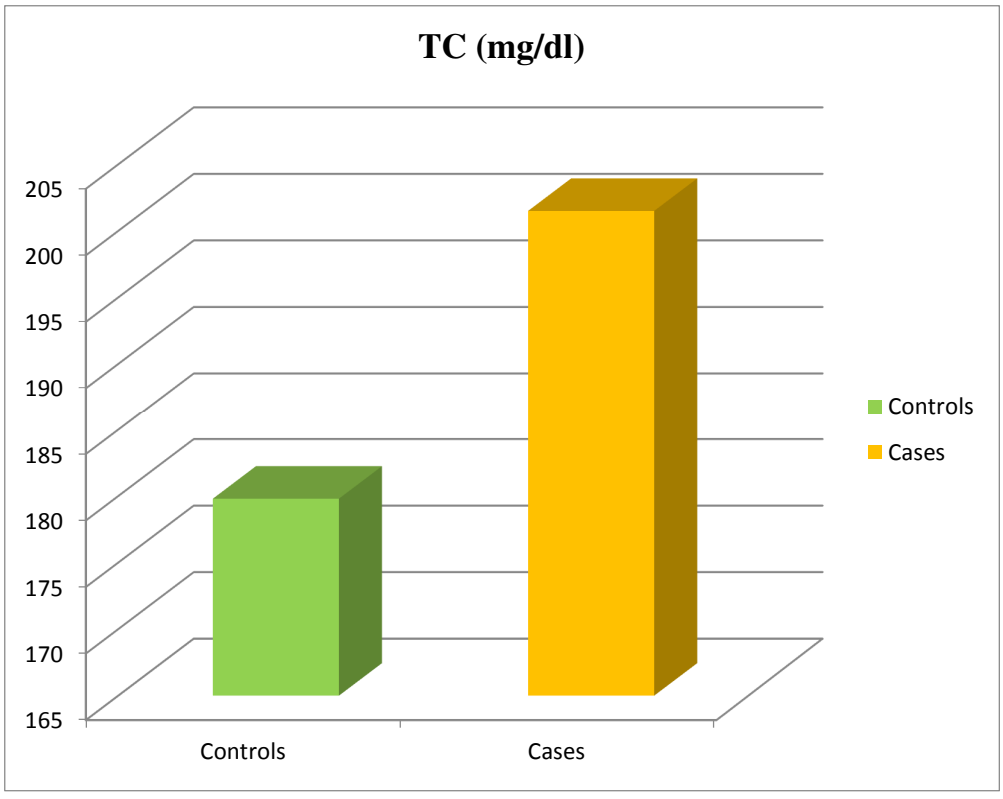


TABLE 9 - Comparison of Serum TGL among cases and controls.

T-TEST			
S.TGL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	132.4	12.6	p value = .0001 <0.05 – Significant
Cases (n=50)	147.7	23.6	

BAR CHART 7

COMPARISON OF SERUM TGL LEVEL BETWEEN CONTROLS AND CASES

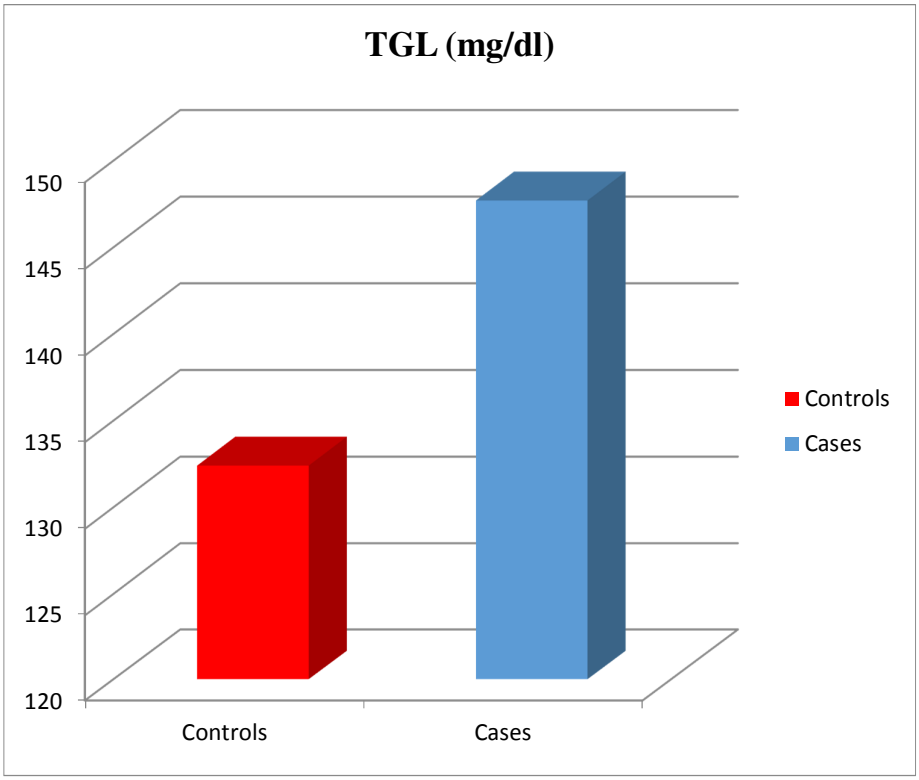


TABLE 10 - Comparison of Serum HDL among cases and controls.

T-TEST			
S.HDL (ng/L)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	38.4	5.7	p value = .0001 <0.05 – Significant
Cases (n=50)	34.7	3.4	

BAR CHART 8

COMPARISON OF SERUM HDL LEVEL BETWEEN CONTROLS AND CASES

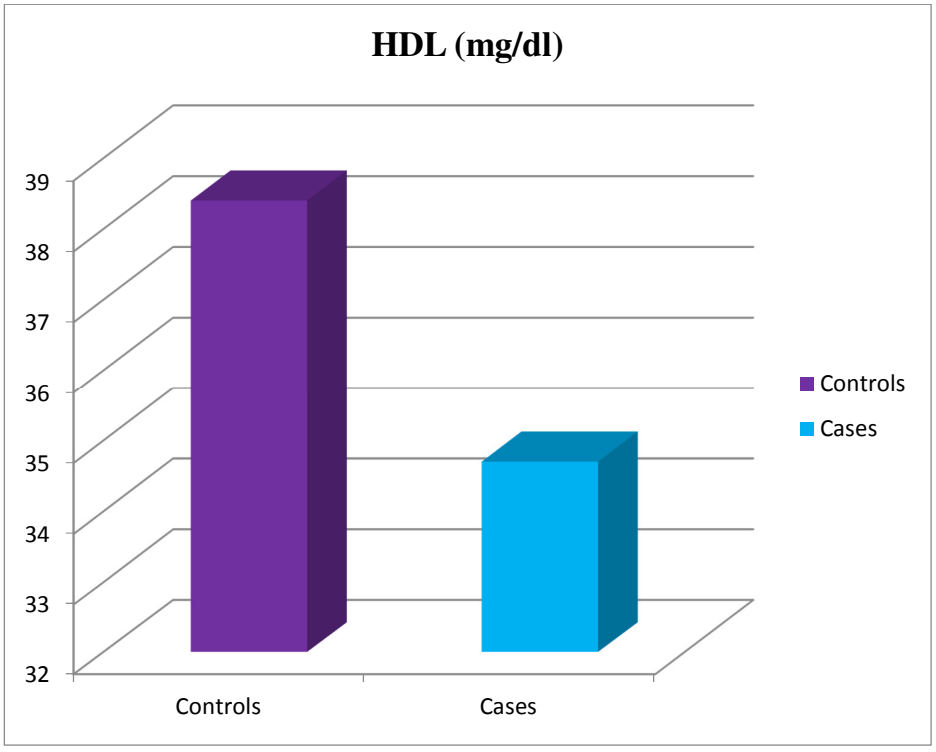


TABLE 11 - Comparison of S.VLDL among cases and controls.

T-TEST			
S.VLDL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	26.7	2.6	p value = .0003 <0.05 – Significant
Cases (n=50)	29.6	4.8	

BAR CHART 9

COMPARISON OF SERUM VLDL LEVEL BETWEEN CONTROLS AND CASES

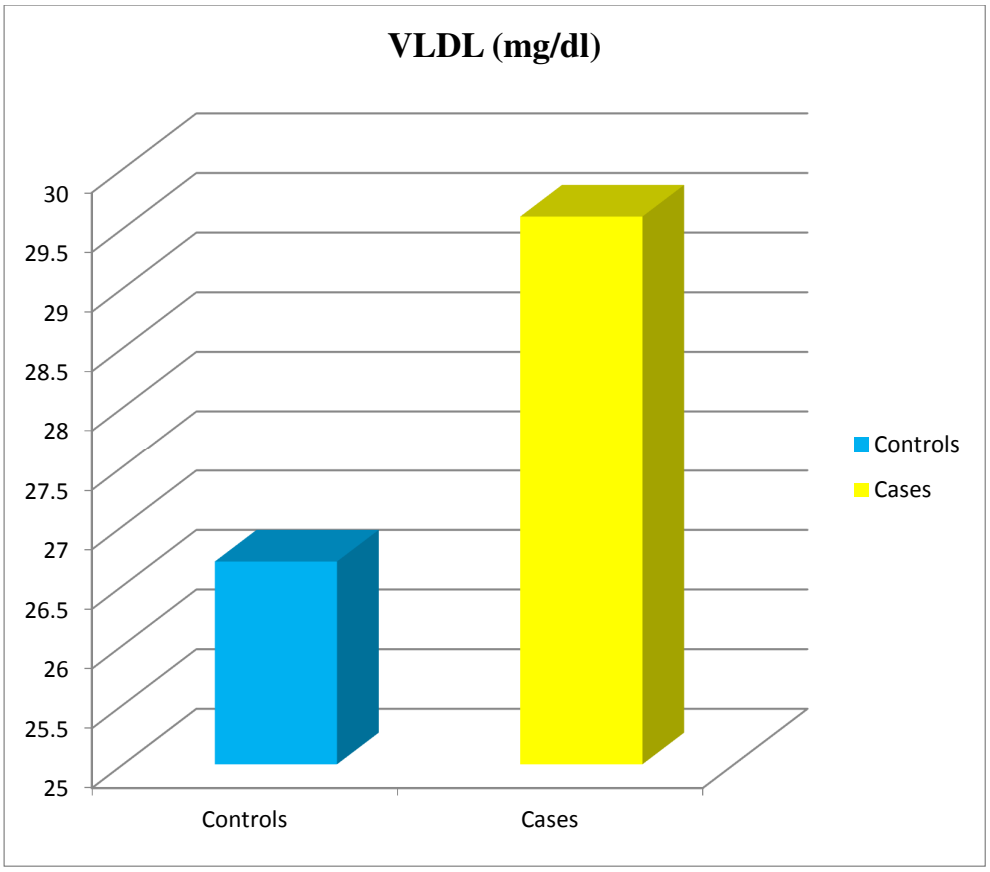


TABLE 12 - Comparison of S.LDL among cases and controls.

T-TEST			
S.LDL (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	115.0	14.0	p value >0.05 – Not Significant
Cases (n=50)	137.0	33.4	

BAR CHART 10

COMPARISON OF SERUM LDL LEVEL BETWEEN CONTROLS AND CASES

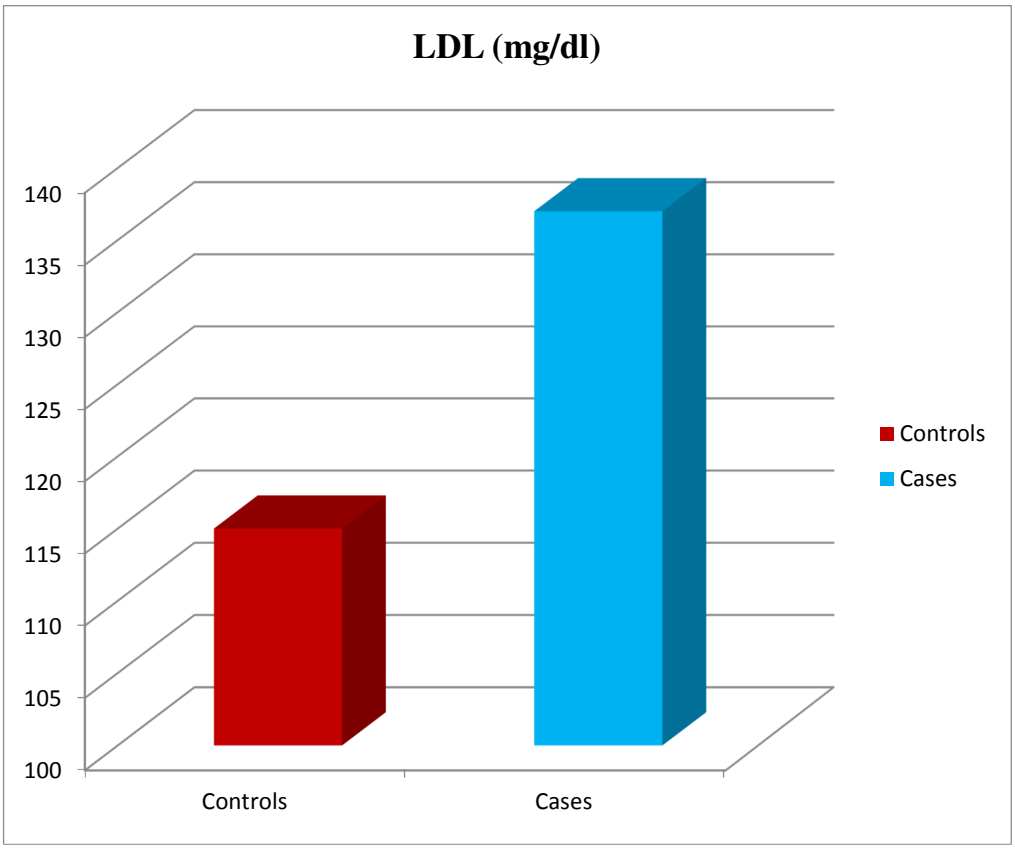


TABLE 13 - Comparison of Height among cases and controls.

T-TEST			
HEIGHT (mt)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	1.61	0.08	p value = .001 <0.05 – Significant
Cases (n=50)	1.67	0.07	

BAR CHART 11

COMPARISON OF HEIGHT BETWEEN CONTROLS AND CASES

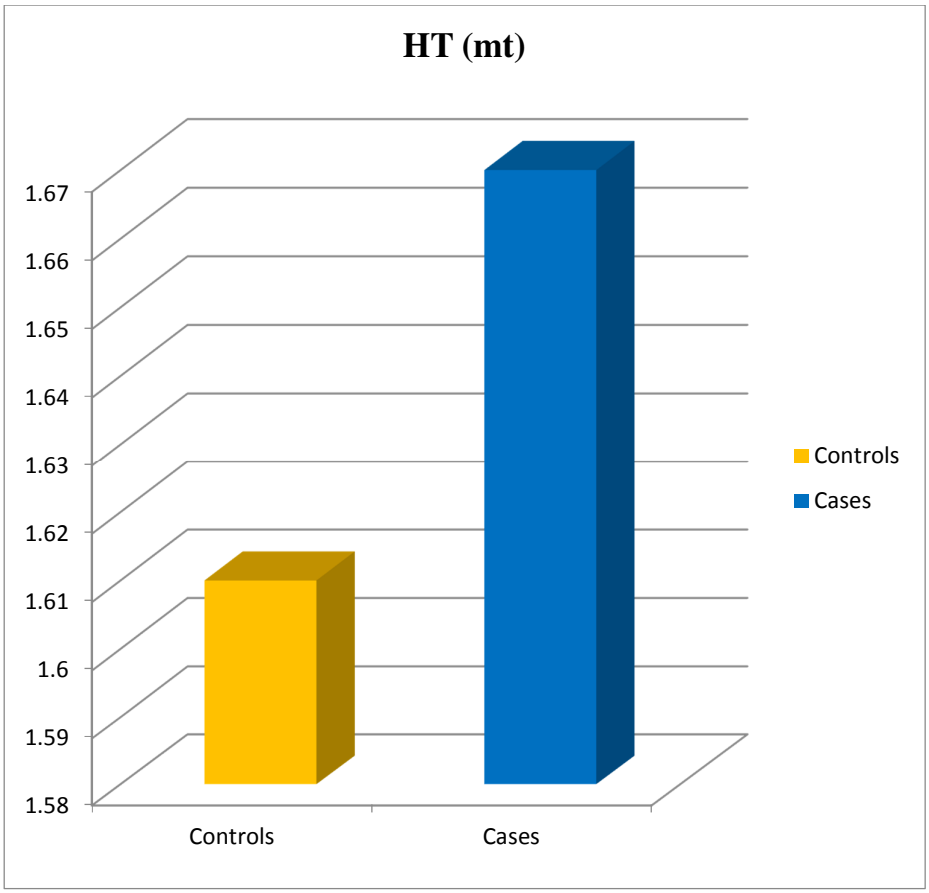


TABLE 14 - Comparison of Weight among cases and controls.

T-TEST			
WEIGHT (Kg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	66.6	7.19	p value = .001 <0.05 – Significant
Cases (n=50)	71.26	7.3	

BAR CHART 12

COMPARISON OF WEIGHT BETWEEN CONTROLS AND CASES

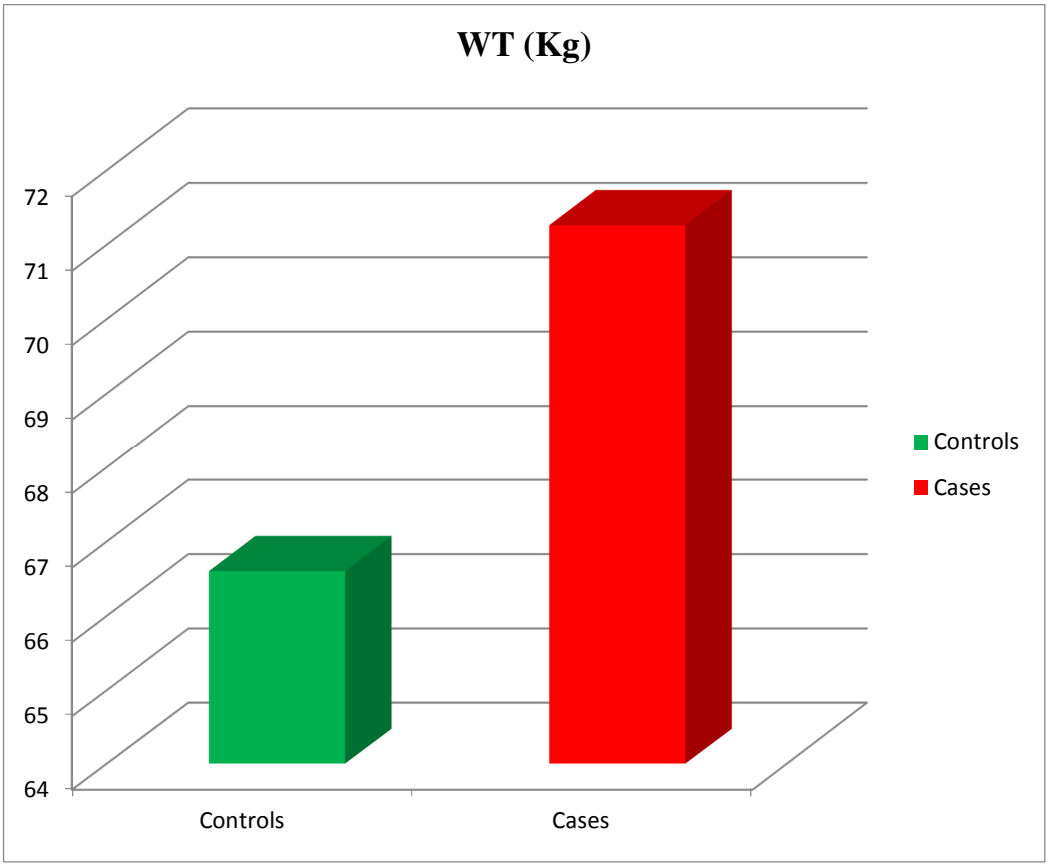


TABLE 15 - Comparison of BMI among cases and controls.

T-TEST			
BMI (Kg/m^2)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	24.82	1.73	p value = >0.05 –Non Significant
Cases (n=50)	25.2	2.87	

BAR CHART 13

COMPARISON OF BMI BETWEEN CONTROLS AND CASES

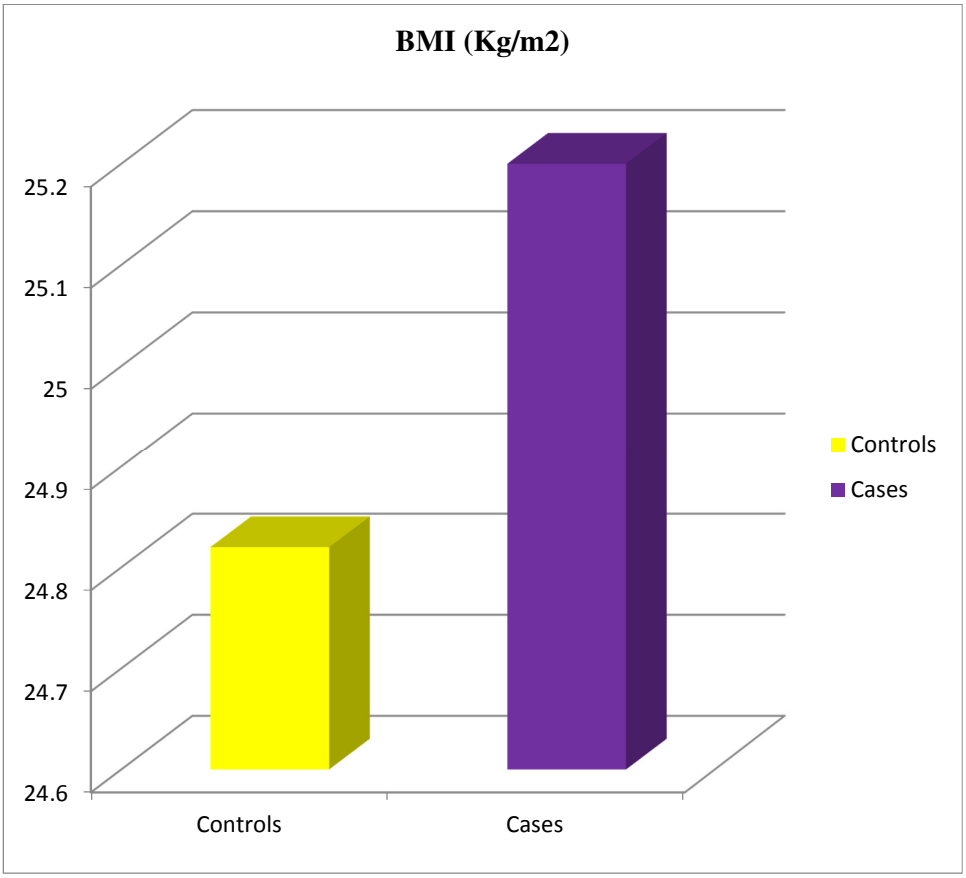


TABLE 16 - Comparison of SBP among cases and controls.

T-TEST			
SBP (mm/Hg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	118	8	p value = .001 <0.05 – Significant
Cases (n=50)	130	26.03	

BAR CHART 14

COMPARISON OF SBP BETWEEN CONTROLS AND CASES

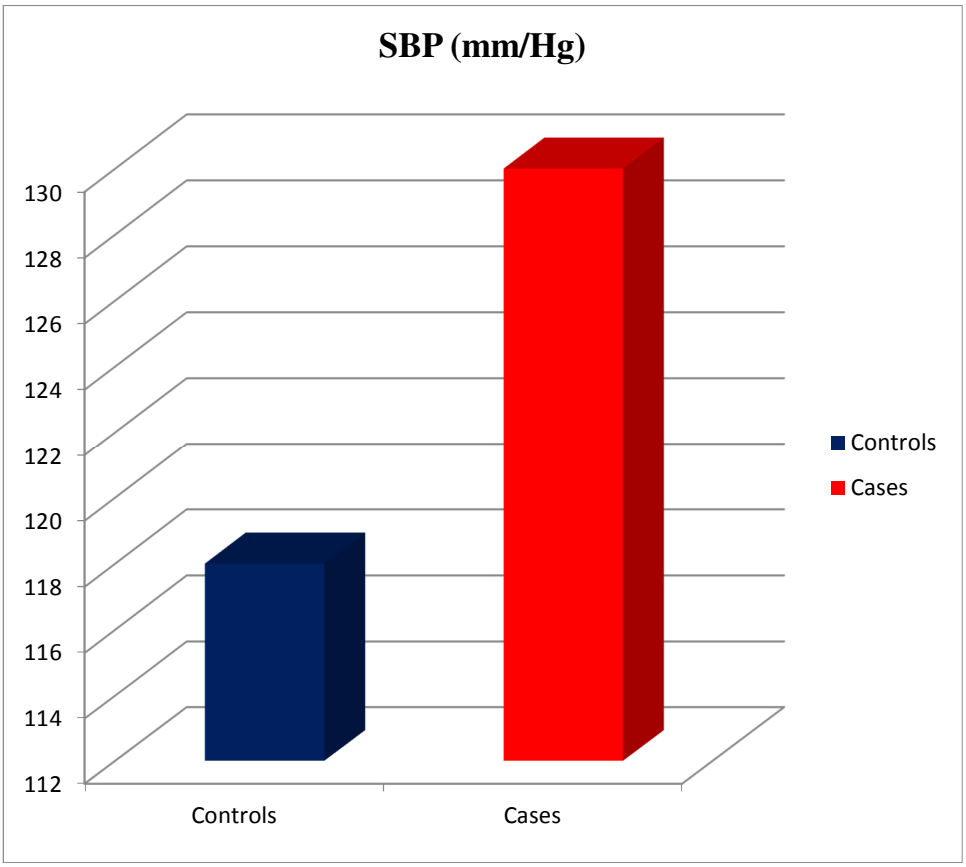


TABLE 17 - Comparison of DBP among cases and controls.

T-TEST			
DBP (mm/Hg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	78	4.46	p value = .001 <0.05 – Significant
Cases (n=50)	84	11.14	

BAR CHART 15

COMPARISON OF DBP BETWEEN CONTROLS AND CASES

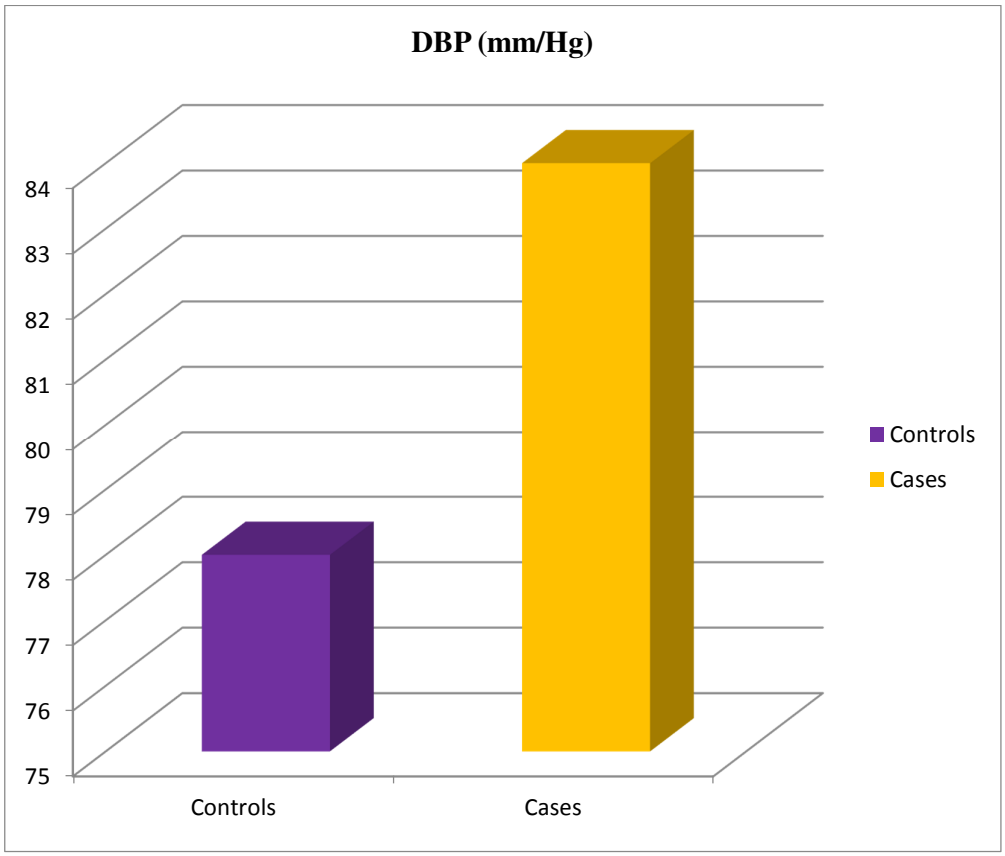


TABLE: 18-Age Distribution among control and cases

AGE	CONTROL(n=50)		CASES(n=50)		TOTAL(n=100)	
41-50 yrs	17	34%	19	38%	36	36%
51-60 yrs	31	62%	30	60%	61	61%
> 60 yrs	2	2%	1	2%	3	3%

BAR CHART 16

AGE DISTRIBUTION

BETWEEN CONTROLS AND CASES

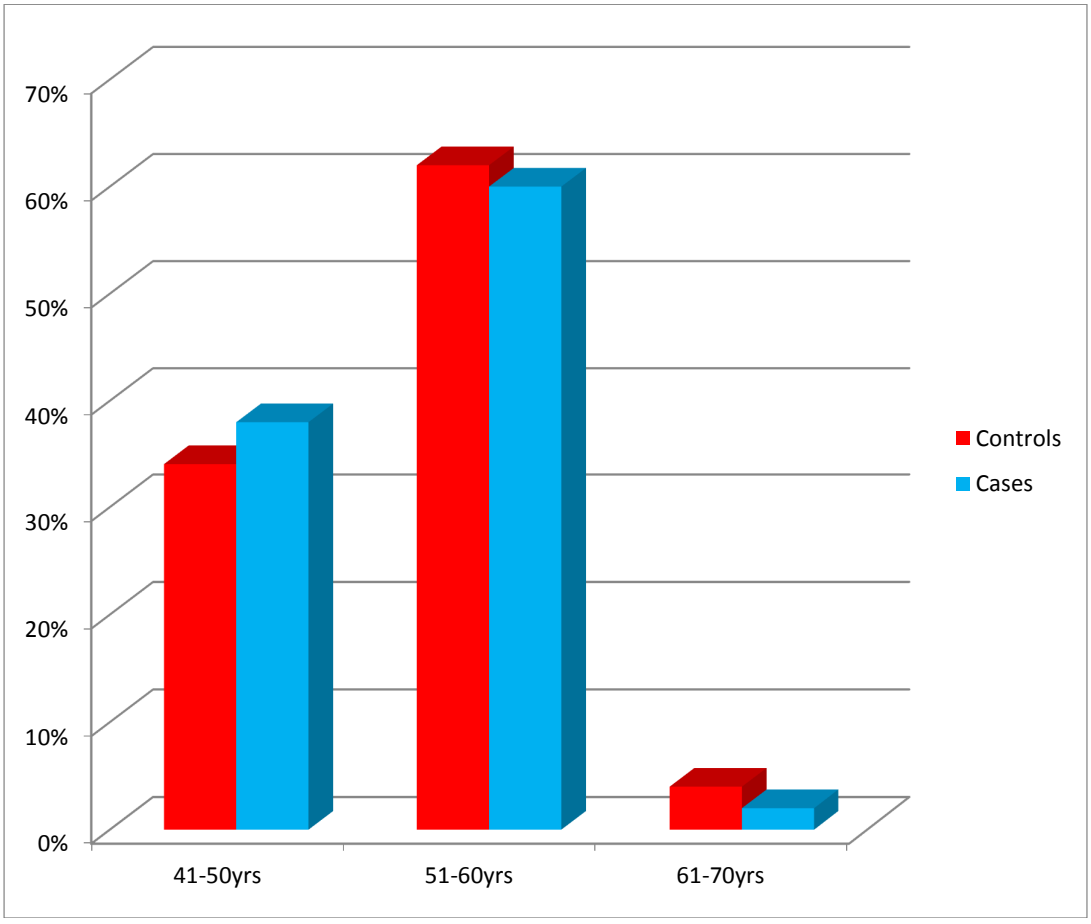


TABLE: 19 Gender Distribution among control and cases.

	CONTROL(n=50)		STUDY(n=50)		TOTAL(n=100)	
Male	**39**	**78%**	**39**	**78%**	**78**	**78%**
Female	**11**	**22%**	**11**	**22%**	**22**	**22%**

BAR CHART 17

GENDER DISTRIBUTION

FOR BOTH CONTROLS AND CASES

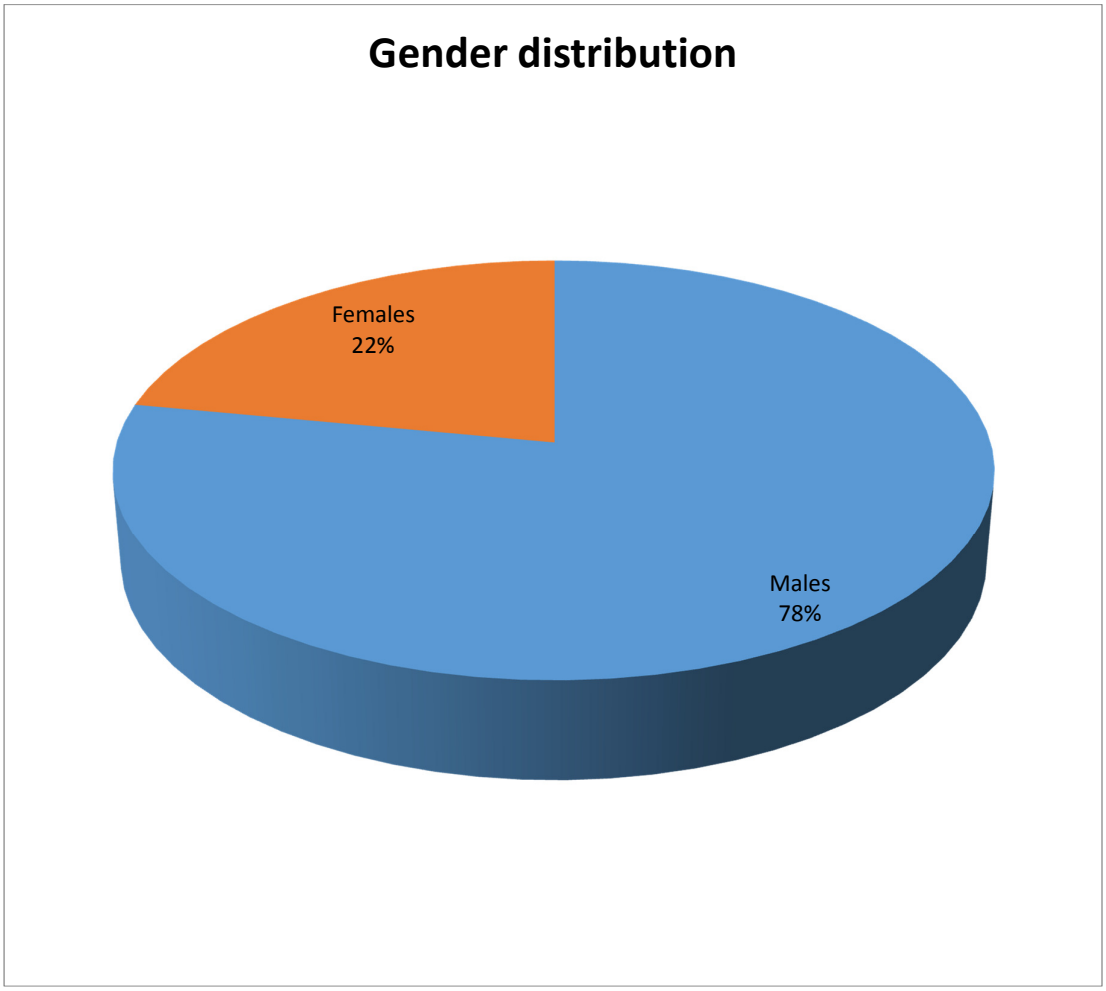


TABLE :20 PEARSON'S CORRELATION BETWEEN S.COPEPTIN AND OTHER PARAMETERS

CASES – COPEPTIN	CORRELATION VALUE	STATISTICAL INFERENCE
CK-MB	+ 0.609	$p < 0.0001$ Significant
RBS	-0.076	$p < 0.001$ Significant
B.UREA	-0.105	$p < 0.0001$ Significant
S.CREATININE	-0.121	$p < 0.0001$ Significant
T.CHOLESTEROL	+0.054	$p < 0.0001$ Significant
TGL	+0.365	$p < 0.0001$ Significant
HDL	+0.192	$p < 0.0001$ Significant
VLDL	+0.345	$p < 0.0003$ Significant
LDL	-0.012	$p > 0.05$ Not Significant
BMI	-0.116	$p > 0.05$ Not Significant
SBP	-0.215	$p < 0.001$ Significant
DBP	-0.074	$p < 0.001$ Significant

STATISTICAL ANALYSIS

- Student's t-test was employed for the statistical analysis of data.
- The data were expressed in terms of mean and standard deviation.
- 'p' value less than 0.05 was taken as the significant value.
- Correlation between the measured parameters was assessed using Pearson's correlation coefficient.

RESULTS

A total of 100 participants were included in the study. Out of these, 50 were grouped under control and 50 were under cases.

The serum value of Copeptin, S.CK-MB, Random blood sugar, B.Urea, S.Creatinine, S.Total Cholesterol, S.HDL and S.TGL were estimated for all the samples in both the groups. BMI, S.VLDL, and S.LDL were calculated. The values obtained for the controls and cases are represented in the master chart I and II respectively.

TABLE: 1

Shows the descriptive base line statistics of the controls and the cases. It includes the mean values of Anthropometric data, Blood Pressure, S.COPEPTIN, S.CK-MB, Random Blood Sugar, B.Urea, S.Creatinine and Lipid Profile.

TABLE: 2

Shows student's t-test analysis of S.COPEPTIN level between cases and controls.

There is increase in the mean S.COPEPTIN in cases (62.5 ± 46.44 ng/L), when compared to the mean S.COPEPTIN in controls (34.8 ± 13.4 ng/L), which is statistically significant. (p value < 0.0001).

TABLE: 3

Shows student's t-test analysis of S.CK-MB between cases and controls.

There is increase in the mean S.CK-MB in cases (16.08± 10.71), when compared to the mean S.CK-MB in controls (9.68± 3.86), which is statistically significant. (p value < 0.0001).

TABLE:4

Shows Scatter diagram represents the correlation between Serum COPEPTIN and time duration in study group.

TABLE: 5

Shows student's t-test analysis of serum RBS between cases and controls.

There is increase in the mean RBS in cases (110.7 ± 35.14), when compared to the mean RBS in controls (93.94± 6.97), which is statistically significant. (p value < 0.001).

TABLE: 6

Shows student's t-test analysis of B.Urea between cases and controls.

There is increase in the mean B.Urea in cases (30.14 ± 7.2), when compared to the mean B.Urea in controls (25.08 ± 5.58), which is statistically significant. (p value < 0.0001).

TABLE: 7

Shows student's t-test analysis of S.Creatinine between cases and controls.

There is increase in the mean S.Creatinine in cases (0.80 ± 0.14), when compared to the mean S.Creatinine in controls (0.71 ± 0.08), which is statistically significant. (p value < 0.0001).

TABLE: 8

Shows student's t-test analysis of serum TC between cases and controls.

There is increase in the mean S.TC in cases (201.5 ±34.9), when compared to the mean S.TC in controls (179.8 ± 14.5), which is statistically significant. (p value < 0.0001).

TABLE: 9

Shows student's t-test analysis of serum TGL between cases and controls.

There is increase in the mean S.TGL in cases (147.7 ± 23.6), when compared to the mean S.TGL in controls (132.4 ±12.6), which is statistically significant. (p value < 0.0001).

TABLE: 10

Shows student's t-test analysis of serum HDL between cases and controls.

There is decrease in the mean S.HDL in cases (34.7 ± 3.4), when compared to the mean S.HDL in controls (38.4 ± 5.7), which is statistically significant.(p value < 0.0001).

TABLE: 11

Shows student's t-test analysis of serum VLDL between cases and controls.

There is increase in the mean S.VLDL in cases (29.6±4.8), when compared to the mean S.VLDL in controls (26.7 ± 2.6), which is statistically significant. (p value < 0.0003).

TABLE: 12

Shows student's t-test analysis of serum LDL between cases and controls.

There is increase in the mean S.LDL in cases (137 ± 33.4), when compared to the mean S.LDL in controls (115 ± 14), which is not statistically significant. (p value > 0.05).

TABLE: 13

Shows student's t-test analysis of Height between cases and controls.

There is increase in the mean Height in cases (1.67 ± 0.07), when compared to the mean Height in controls (1.61 ±0.08), which is statistically significant.

(p value < 0.001).

TABLE: 14

Shows student's t-test analysis of Weight between cases and controls.

There is increase in the mean Weight in cases (71.26 ± 7.3), when compared to the mean Weight in controls (66.6 ± 7.19), which is statistically significant. (p value < 0.001).

TABLE: 15

Shows student's t-test analysis of BMI between cases and controls.

There is increase in the mean BMI in cases (25.2 ± 2.87), when compared to the mean BMI in controls (24.82 ± 1.73), which is not statistically significant. (p value >0.05).

TABLE: 16

Shows student's t-test analysis of Systolic BP between cases and controls.

There is increase in the mean Systolic BP in cases (130 ± 26.03), when compared to the mean Systolic BP in controls (118 ± 8), which is statistically significant. (p value < 0.001).

TABLE: 17

Shows student's t-test analysis of Diastolic BP between cases and controls.

There is increase in the mean Diastolic BP in cases (84 ± 11.14), when compared to the mean Diastolic BP in controls (78 ± 4.46), which is statistically significant. (p value < 0.05).

TABLE: 18

Shows the age distribution among cases and controls.

The mean age falls between 51.88 ± 4.50.

TABLE: 19

Shows gender distribution in both control and study group. Among the patients taken in this study 78% were males and 22% were females.

TABLE: 20

Pearson's correlation between S.COPEPTIN and other parameters.

This table shows positive correlation between S.COPEPTIN and CK-MB, which are statisfically significant ($p < 0.0001$).There was no significant correlation of S.COPEPTIN with RBS, B.Urea, S.Creatinine, T.Cholesterol, S.TGL, S.HDL, S.VLDL, S.LDL, BMI, SBP &DBP.

DISCUSSION

In this study,the mean value of serum COPEPTIN (62.5 $\pm$ 46.44 ng/L) was significantly higher than that of healthy controls (34.8 $\pm$ 13.4 ng/L).The mean S.CK-MB (16.08 $\pm$ 10.71) level in the study group was significantly higher than in control group (9.68 $\pm$ 3.86). But, CK-MB did not increase significantly as S.COPEPTIN increased in the early hours of AMI. Pearson's correlation analysis also showed significant correlation between S.COPEPTIN with S.CK-MB.

The mean S.total cholesterol level in the study group (201.5 ± 34.9) was higher than the control group (179.8 ± 14.5) which was statistically significant. The mean serum HDL-C which is lower in the study group compared to the control group (34.7± 3.4 versus 38.4 ± 5.7) which was statistically significant ($p < 0.0001$).The mean values of S.LDL-C, S.VLDL, and S.TGL are also significantly increased in the study group than the control group .

The role of S.COPEPTIN has gained attention particularly in patients with AMI. Circulating levels of S.COPEPTIN is significantly increased during acute phase of AMI when compared to healthy controls.

The stimuli for COPEPTIN release after AMI is acute endogenous stress. COPEPTIN release is also related to changes in fluid volume.Thus,hemodynamic changes occurring in acute phase during AMI may trigger COPEPTIN release. COPEPTIN is the C-terminal part of pro AVP. So, COPEPTIN and AVP share the

common precursor peptide. The hemodynamic changes in AMI causes release of AVP as well as COPEPTIN. Unlike AVP ,the COPEPTIN is relatively stable in blood circulation and methodologically it is easier to determine.

Myocardial necrosis directly leads to COPEPTIN release from the heart. In contrast to S.CK-MB & Cardiac Troponin, S.COPEPTIN rises immediately after the onset of symptoms.A direct relationship between level of S. COPEPTIN and size of infarct has been demonstrated in ACS patients.

Reichlin and Muller demonstrated the role of S.COPEPTIN in the management of 487 consecutive patients with chest pain admitted in the hospital.The serum levels of COPEPTIN were found to be increased within 0 to 4 hours after the onset of symptoms, when troponin T was still undetectable in most of the patients.[93]

Elevation in S.COPEPTIN identifies the AMI ,before the onset of myocardial necrosis and are well correlated with S.CK-MB levels also. The levels of S.COPEPTIN is significantly higher in AMI patients than the healthy group and these results are in compliance with many literature data.

As in table 4,the scatter diagram of this study revealed

1. The significant increase of S.COPEPTIN within 4 hrs of chest pain when compared to S.CK-MB
2. The direct relation ship between S.COPEPTIN level and time.

So, the estimation of S.COPEPTIN can be used to diagnose AMI at the earlier stage.

CONCLUSION

Biochemical markers such as CK-MB, Cardiac Troponin-I and Myoglobin are suitable for diagnosing myocardial infarction. However,elevation of these markers indicate myocardial necrosis and in the absence of necrosis these markers are not elevated.

This study shows that serum levels of COPEPTIN are high in patients with AMI at very early stage.

COPEPTIN is a new bio marker of acute endogenous (haemodynamic) stress. In AMI, which is an acute stressful state, S.COPEPTIN level rises**.** Measurement of S.COPEPTIN helps to diagnose AMI in Emergency Department before the onset of necrosis.. Measuring S.COPEPTIN along with ECG and other markers improves the diagnostic sensitivity of the method.

S.COPEPTIN can be used as a novel early marker in patients with AMI. Hence, earlier diagnosis helps in reducing the morbidity and mortality from AMI.

FUTURE SCOPE OF THE STUDY

Estimation of S.COPEPTIN in outpatient department can be done in patients with chest pain to rule out AMI before ECG manifestations.

It can be also used to rule out non anginal causes of chest pain. If these observations are confirmed, S.COPEPTIN can be used as an outpatient investigation tool, to reduce inappropriate Hospital Admissions of Low risk patients.

LIMITATIONS OF THE STUDY

This study had the following limitations :

1. The sample size was small.
2. This assay needs to be evaluated by incorporating it into decision making algorithm in an emergency department.
3. Other valuable relevant markers like Cardiac Troponin T are not included in the study.

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A STUDY OF SERUM COPEPTINLEVELIN ACUTE MYOCARDIAL INFARCTION

PROFORMA

NAME OF THE PATIENT :

AGE :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

GENERAL EXAMINATION:

HT: WT: BMI:

BP:

SYSTEMIC EXAMINATION:

CVS: RS:

ABD: CNS:

INVESTIGATIONS :

1.SERUM COPEPTIN:

2.SERUM CREATINE KINASE-2[CK-MB]:

3.LIPID PROFILE

TOTAL CHOLESTEROL:

TGL:

HDL-C:

LDL-C:

VLDL:

4. RANDOM BLOOD SUGAR:

5. BLOOD UREA:

6. SERUM CREATININE:

CONSENT FORM

Dr.**S.SASIKALA,**Post Graduate student in the department of Biochemistry, Thanjavur medical college, Thanjavur is doing ' **A study of serum Copeptin level in Acute Myocardial Infarction'.** The procedure has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature:

Name:

Place: